

Insights into the morphometric and molecular characterisation of ber fruit fly (*Carpomyia vesuviana* Costa) infesting jujube ber, *Ziziphus mauritiana* (Lamk.)

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Citation: Kavin P., Usharani B., Kamala Jayanthi P.D., Suresh K., Gracy G., Singh S., Kennady R.N., Mini M.L., Ramessh C. (2025): Insights into the morphometric and molecular characterisation of ber fruit fly (*Carpomyia vesuviana* Costa) infesting jujube ber, *Ziziphus mauritiana* (Lamk.). Plant. Protect. Sci., 61: 350–365.

Abstract: *Carpomyia vesuviana* Costa, a fruit fly species, is a major pest affecting the jujube (Ber). This monophagous insect pest causes significant economic losses in regions where the jujube is cultivated. Hence, the present study was conducted to provide a detailed morphometric and molecular analysis of *C. vesuviana* across various Indian regions, specifically Tamil Nadu, Punjab, and Rajasthan. Morphometric measurements were conducted for various developmental stages, from the egg to adult. The overall mean length and width of the egg were 0.61 mm and 0.18 mm, respectively, and the fully grown maggot measured 6.19 mm (length) and 1.91 mm (width). The length and width of the prepupa and pupa were 7.67 mm and 1.64 mm, and 4.00 mm and 1.72 mm, respectively. The overall mean length of the female whole body and wing expanse measured 4.74 mm and 3.92 mm, respectively. The overall mean male whole body length and wing expanse were 4.19 mm and 3.56 mm, respectively. The results of the Principal Component Analysis (PCA) revealed that the maggot's length at different stages, pupa length, and egg width were the main contributors to the variability, particularly in the samples from Rajasthan. Additionally, the adult female fly's morphometric traits, such as the wing and thorax measurements, showed regional variations, with the PCA highlighting Punjab's alignment with larger head and abdomen traits. The molecular analysis based on PCR and sequencing of the COXI–COXII region confirmed the species identity. The nucleotide sequence of *C. vesuviana* from Tamil Nadu and Punjab was deposited in GenBank as PQ198003.1 and PQ198005.1 which had nearly 99% genetic similarity with two sequences NC_071721.1 (Beijing, China) and MT121231.1 (Beijing, China) submitted in the NCBI database. A phylogenetic analysis further demonstrated that the Tamil Nadu and Punjab populations were closely related to an Iranian sample, while samples from other countries, such as the *C. schineri*

of the USA and *C. incompleta* of Iraq, exhibited higher genetic divergence. The findings underline both morphological and genetic variations within *C. vesuviana*, reflecting the geographical influence on the species development and evolution.

Keywords: ber; DNA barcoding; morphometrics; molecular and principal component analysis

Jujube (ber) fruit fly (JFF), *Carpomyia vesuviana* Costa (Tephritidae; Diptera) is an economically important pest infesting only the host of the jujube *Ziziphus sp.* This genus of fruit fly includes *C. vesuviana*, *C. incompleta* Becker, 1903, *C. schineri* Loew, 1856, *C. liat* Freidberg, 2016, *C. weidmanni* Meigen, 1826, *C. paradalina* Bigot, 1891 and *C. tica* Norrborn, 1997. In India, Gujarat, Maharashtra, Haryana, Punjab, Rajasthan and Maharashtra are the main cultivated areas of the jujube (Haldhar et al. 2016). However, ber cultivation in various parts of the county is under threat by fruit flies which have caused yield losses of 80 % in severe conditions (Batra 1953) and they have infested different species of ber.

The adult fruit flies lay their single eggs at the onset of immature fruits with a protractible ovipositor just beneath the outer surface of the fruit. When the maggots hatch and start feeding on the pulp and create galleries with accumulated excreta, which causes the fruit to rot and taste bitter (Bagdavadze 1977). Infested fruits swell up and are oversized and, in extreme situations, they even fall off (Lakra 1998; Singh et al. 2020; Gupta & Sharma 2006). According to Bagle (1992) and Joshi & Shinde (1971), the occurrence of *C. vesuviana* could reduce the yield by 13–100%, respectively in various parts of India. In Northern India, the infestations happen between November and April, with a higher peak of activity in fruit maturation. Lakra and Singh (1983) reported that there were six to nine overlapping generations per year. The incidence was highest in December and lowest in March. The findings of Sohi and Sandhu (1990) reported that the pupae hibernates in the soil from April to August, which causes the fly activity during off season fruit in Punjab.

Preliminary research was conducted through a roving survey from April 2023 to March 2024 across various blocks of Tamil Nadu, including the Aruppukottai block in Virudhunagar District, Kottampatti block in Madurai District, Vazhavacha-

nur block in Tiruvannamalai District and Killikulam block in Tirunelveli District to document fruit fly infestations in the ber agroecosystem. The results revealed that irrespective of the surveyed location, the fruit fly *C. vesuviana* contributed to yield losses ranging 40–70%. The highest infestation was recorded in the Aruppukottai region, where losses peaked at 70% with the severity being most pronounced during January. In the Madurai region, the infestation level was comparatively lower accounting for around a 40% yield loss.

Being a monophagous and potential pest on the jujube, comprehensive research on its morphology, as well as its morphometric and genetic traits are highly essential. Hence, the unexplored characteristics of *C. vesuviana*, such as its taxonomic identification with molecular and morphometric characteristics, were carried out in this study

MATERIAL AND METHODS

Sample Collection. Fruit-fly infested samples were collected from jujube orchards at the Regional Research Station, Aruppukottai, Virudhunagar District, Tamil Nadu, ber orchards at Punjab Agricultural University, Ludhiana, Punjab and orchards at ICAR – Central Institute for Arid Horticulture, Bikaner (Rajasthan). The infested fruit samples were kept in separate rearing cages and the adults that emerged were collected and preserved in 70% ethanol. To identify the specimens, the samples were sent to Dr. K. J. David, Professor (Ento.) and Senior Scientist at ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Hebbal (Bengaluru). The insects were morphologically identified using the *Carpomyini* taxonomical key (Korneyev et al. 2017). Morphometric measurements of various body parts were carried out from ten insect samples, conducted with a LEICA M205A, Software Leica Application Suite (LASX) (version 4.12) stereoscopic microscope fitted with a camera at the Centre for Plant Protection Studies,

Tamil Nadu Agriculture University, Coimbatore and Centre of Innovation at Agricultural College and Research Institute, Madurai.

Molecular Identification. One insect was taken for the molecular identification from each location, such as Tamil Nadu and Punjab. This work was performed at the National Professor Lab at ICAR – Indian Institute of Horticultural Research, Bengaluru. The DNA extraction was conducted by using the Cetyltrimethylammonium bromide (CTAB) method. Samples were crushed in a mortar with a pestle by adding a sufficient amount of liquid nitrogen. The crushed samples were transferred into a 2 mL microcentrifuge tube with 700 μ L of the CTAB buffer. The samples were incubated at 60 °C for 30–45 min. Then, 700 μ L of each tube mix was added gently and kept for centrifugation at 13 000 rpm for 10 min. The clear upper solution was taken in a new 2 mL microcentrifuge tube and the lower debris was discarded. Phenol:Chloroform:Isoamyl alcohol (P:C:I) (25:24:1) equal volume mix was added gently and kept it for centrifugation at 13 000 rpm for 10 min. The supernatant was pipetted in a 1.5ml microcentrifuge tube, to that sodium acetate (3M, 5.7pH) and an equal

volume of iso propanol was added and kept it overnight for incubation for the duration of 30 min. Centrifuged at 13 000 rpm for 15 min 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. 3 μ L of RNase was added to that and kept it for 15 min at ambient temperature. Incubation was carried out at 37 °C and then 55 °C for 30 s and stored it 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'-TAAACTTCAGGGT-GACCAAAAAATCA-3') (Folmer et al. 1994). Polymerase chain reaction (PCR) amplification was carried out using the primers LCO1490 and HC02198 following the protocol described by Folmer et al. (1994). The process included an initial denaturation stage, followed by denaturation, annealing, and extension. A final extension stage 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 the mitochondrial genome. The prim-

Table 1. Morphometrics of *Carpomyia vesuviana* (immature stage) from different locations in India

S. No.	Characters	Tamil Nadu		Punjab		Rajasthan		Overall mean (mm)
		Range (mm)	Mean $\pm$ Std (mm)	Range (mm)	Mean $\pm$ Std (mm)	Range (mm)	Mean $\pm$ Std (mm)	
1		Egg						
	Length	0.59–0.54	0.58 $\pm$ 0.02	0.56–0.49	0.54 $\pm$ 0.03	0.76–0.67	0.72 $\pm$ 0.03	0.61 $\pm$ 0.09
	Width	0.17–0.15	0.16 $\pm$ 0.01	0.21–0.16	0.19 $\pm$ 0.02	0.21–0.16	0.19 $\pm$ 0.02	0.18 $\pm$ 0.02
2		Maggots –3 rd day after hatching from egg						
	Length	4.86–4.71	4.81 $\pm$ 0.05	4.91–4.79	4.84 $\pm$ 0.04	4.83–4.71	4.77 $\pm$ 0.05	4.81 $\pm$ 0.03
	Width	1.38–1.27	1.33 $\pm$ 0.03	1.39–1.32	1.36 $\pm$ 0.03	1.47–1.36	1.41 $\pm$ 0.03	1.36 $\pm$ 0.04
3		Maggots –5 th day after hatching from egg						
	Length	5.41–5.29	5.35 $\pm$ 0.04	5.56–5.17	5.41 $\pm$ 0.11	5.59–5.41	5.51 $\pm$ 0.06	5.42 $\pm$ 0.08
	Width	1.53–1.46	1.51 $\pm$ 0.02	1.55–1.44	1.52 $\pm$ 0.05	1.59–1.46	1.54 $\pm$ 0.05	1.52 $\pm$ 0.02
4		Maggots –8 th day after hatching from egg						
	Length	6.28–6.11	6.18 $\pm$ 0.05	6.33–6.21	6.27 $\pm$ 0.04	6.26–6.04	6.14 $\pm$ 0.07	6.19 $\pm$ 0.07
	Width	1.91–1.78	1.88 $\pm$ 0.04	1.97–1.89	1.94 $\pm$ 0.03	1.96–1.86	1.92 $\pm$ 0.03	1.91 $\pm$ 0.03
5		Pre pupa						
	Length	7.58–7.45	7.52 $\pm$ 0.05	7.71–7.61	7.66 $\pm$ 0.03	7.89–7.78	7.84 $\pm$ 0.04	7.67 $\pm$ 0.16
	Width	1.67–1.55	1.62 $\pm$ 0.03	1.71–1.56	1.63 $\pm$ 0.05	1.69–1.62	1.65 $\pm$ 0.03	1.64 $\pm$ 0.02
6		Pupa						
	Length	4.27–4.06	4.17 $\pm$ 0.06	4.17–3.79	3.92 $\pm$ 0.13	4.04–3.84	3.92 $\pm$ 0.07	4.00 $\pm$ 0.15
	Width	1.79–1.65	1.71 $\pm$ 0.05	1.77–1.66	1.73 $\pm$ 0.04	1.78–1.67	1.74 $\pm$ 0.04	1.72 $\pm$ 0.01

n =10 replications were taken with each location

ers were prepared at a concentration of 10 picomol/mL, according to manufacturer's instructions. The PCR reaction was carried out using a reaction mixture of 50 µL of the reaction components, 25 µL of the prepared reaction mixture [TaqDNA Polymerase Master Mix (Amplicon, Denmark)], 0.25 µL of each forward and reverse primer, and 3.5 µL of water. The amplification was performed using a thermal cycler (Techne TC-3000X ThermalCycler, Bibby Scientific Ltd, UK) according to the thermal cycling program mentioned by Folmer et al. (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., India) for reading the sequences of the nitrogenous bases for each sample.

The sequence processing was performed using BioEdit (version 7.0). The processed sequences

were compared with the deposited sequences in the US National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) to determine the identification, which was based on the maximum score and query cover, and the percentage identity provided by the tool with the highest values of the criteria used to confirm the identification at the species level. After confirming the identification, the identified sequences were deposited and registered in the NCBI GenBank® [<https://www.ncbi.nlm.nih.gov/>] (accessed on September 10, 2024)]. The sequence homology was interpreted by comparing other *C. vesuviana*; *C. schineri* and *C. incompleta* sequences with the NCBI Gen Bank database. *C. schineri* was the sub species of this *Carpomyia* sp.

Evolutionary relationships of the taxa. The evolutionary history was inferred using the Neighbour-joining method (Saitou & Nei 1987). The percent-

Table 2. Morphometrics of *Carpomyia vesuviana* (female adult) from different locations in India

S. No.	Characters	Tamil Nadu		Punjab		Rajasthan		Overall mean (mm)
		Range (mm)	Mean $\pm$ Std (mm)	Range (mm)	Mean $\pm$ Std (mm)	Range (mm)	Mean $\pm$ Std (mm)	
1		Antenna						
	Length	0.59–0.49	0.55 $\pm$ 0.03	0.59–0.56	0.57 $\pm$ 0.01	0.58–0.47	0.53 $\pm$ 0.04	0.55 $\pm$ 0.02
2		Head						
	Length	1.55–1.45	1.51 $\pm$ 0.04	1.58–1.48	1.53 $\pm$ 0.03	1.56–1.41	1.48 $\pm$ 0.05	1.50 $\pm$ 0.03
	Width	1.55–1.41	1.47 $\pm$ 0.04	1.51–1.46	1.48 $\pm$ 0.02	1.49–1.41	1.46 $\pm$ 0.02	1.47 $\pm$ 0.01
3		Thorax						
	Length	1.97–1.89	1.93 $\pm$ 0.02	1.95–1.89	1.91 $\pm$ 0.02	1.97–1.86	1.89 $\pm$ 0.03	1.91 $\pm$ 0.02
	Width	1.56–1.33	1.47 $\pm$ 0.06	1.59–1.46	1.51 $\pm$ 0.04	1.48–1.39	1.44 $\pm$ 0.03	1.47 $\pm$ 0.04
4		Leg						
	Fore leg	1.16–1.12	1.13 $\pm$ 0.01	1.14–1.07	1.11 $\pm$ 0.02	1.16–1.09	1.14 $\pm$ 0.02	1.13 $\pm$ 0.02
	Mid leg	1.31–1.27	1.29 $\pm$ 0.01	1.28–1.19	1.23 $\pm$ 0.03	1.31–1.21	1.27 $\pm$ 0.03	1.26 $\pm$ 0.03
	Hind leg	1.63–1.49	1.55 $\pm$ 0.04	1.53–1.48	1.51 $\pm$ 0.02	1.59–1.49	1.53 $\pm$ 0.03	1.53 $\pm$ 0.02
5		Wing						
	Length	3.99–3.67	3.87 $\pm$ 0.09	3.96–3.87	3.93 $\pm$ 0.03	3.95–3.84	3.90 $\pm$ 0.03	3.90 $\pm$ 0.03
	Width	1.49–1.34	1.43 $\pm$ 0.05	1.47–1.39	1.44 $\pm$ 0.02	1.49–1.41	1.46 $\pm$ 0.03	1.44 $\pm$ 0.02
6		Abdomen						
	Length	1.91–1.81	1.85 $\pm$ 0.03	1.96–1.79	1.88 $\pm$ 0.05	1.93–1.83	1.88 $\pm$ 0.03	1.87 $\pm$ 0.01
	Width	1.66–1.56	1.62 $\pm$ 0.03	1.73–1.64	1.69 $\pm$ 0.03	1.68–1.62	1.65 $\pm$ 0.02	1.65 $\pm$ 0.03
7		Ovipositor						
	Length	0.39–0.28	0.33 $\pm$ 0.03	0.37–0.29	0.34 $\pm$ 0.03	0.41–0.31	0.36 $\pm$ 0.03	0.34 $\pm$ 0.02
8		Whole body						
	Length	4.74–4.71	4.73 $\pm$ 0.02	4.89–4.66	4.75 $\pm$ 0.08	4.79–4.71	4.76 $\pm$ 0.03	4.74 $\pm$ 0.01
	Wing expanse	3.96–3.89	3.94 $\pm$ 0.02	3.94–3.84	3.90 $\pm$ 0.04	3.98–3.86	3.93 $\pm$ 0.05	3.92 $\pm$ 0.02

n = 10 replications were taken with each location

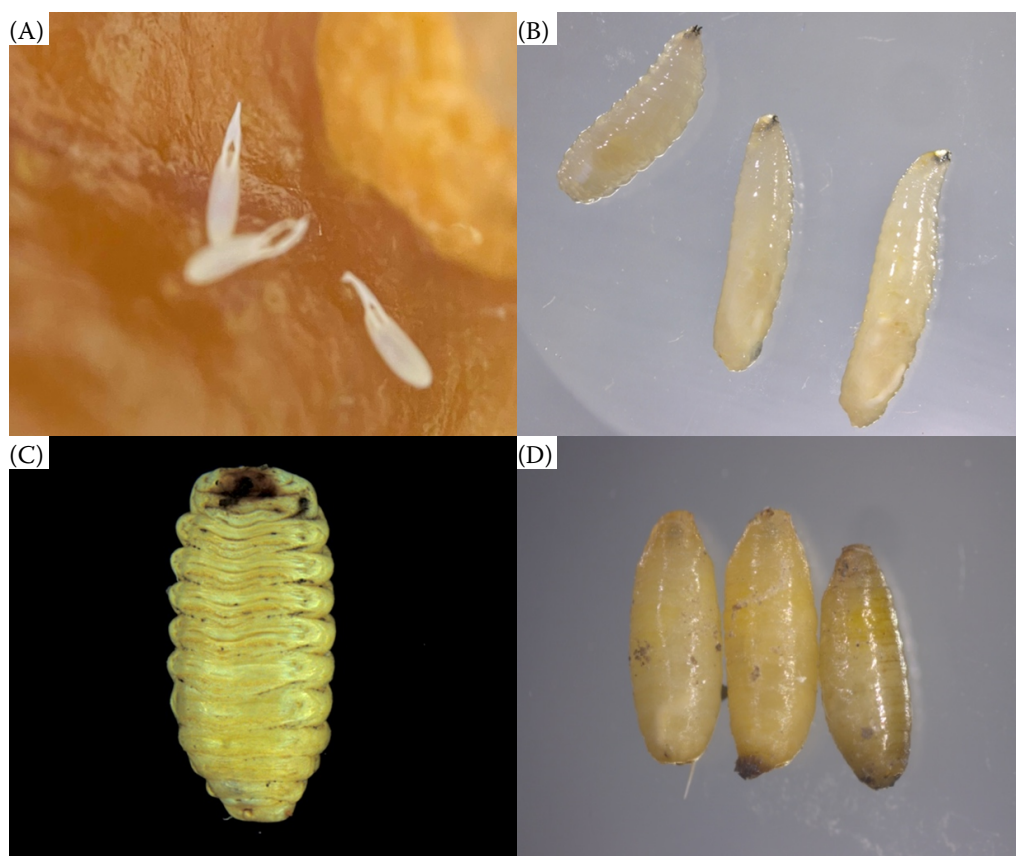


Figure 1. Morphological features of the immature stages of the ber fruit fly, *Carpomyia vesuviana* (Tephritidae; Diptera) A – eggs are small, elongate, spindle shaped and creamy white colored; B – maggots are worm shaped, translucent and creamy white; C – pre-pupa are become maggot was creamy white, started feeding on pulp; D – pupa are creamy white colour later turn into brown and barrel shaped

age of replicate trees in which the associated taxa clustered together in the bootstrap test (1 000 replicates) is shown on the branches (Felsenstein 1985). Evolutionary distances were computed using the maximum composite likelihood method (Tamura et al. 2004) and are expressed in terms of the number of base substitutions per site. This analysis involved twelve nucleotide sequences, and all the ambiguous positions were removed for each sequence pair using the pairwise deletion option. A total of 711 positions were present in the final dataset. The evolutionary analyses were conducted in MEGA software (version 11) (Stecher et al. 2020; Tamura et al. 2021).

Statistical analysis. All the statistical analyses to determine the impact of the different geographical locations on the morphometric variations and graphical representation were performed in open source R software [<http://www.rstudio.com> accessed on September 12, 2024] (Team R 2015). The Principal Component Analysis (PCA) of the

different life stages of *C.vesuviana* was performed by using the "Factoextra" (Kassambara & Mundt 2017) and "Factominer" (Husson et al. 2013) packages. The biplot of the PCA was also obtained by using the package Grammar of Graphics in R Studio (ggplot2 package).

RESULTS

The species was morphologically identified as *C. vesuviana* Costa. The results of the morphometrics measurements of *C. vesuviana* are given Tables 1, 2 and 3. With regards to the morphometrics of the immature stage of JFF, the mean length and width of the eggs from Tamil Nadu, Punjab and Rajasthan measured 0.58 mm and 0.16 mm, 0.54 mm and 0.19 mm, 0.72 mm and 0.19 mm, respectively. The eggs were laid in the cavities made by the female's protrusible ovipositor, just beneath the skin of the fruit which is illustrated in Figure 3C.

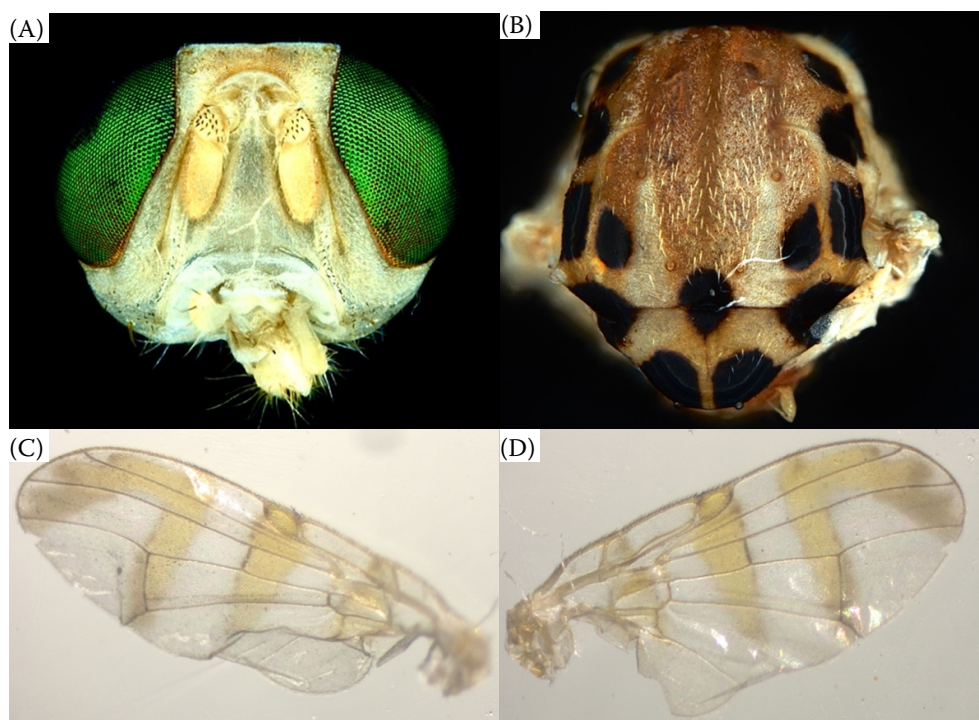


Figure 2. Morphological features of adult characters of fruit fly, *Carpomyia vesuviana* (Tephritidae; Diptera)
 A – head and ommatidia, the compound eyes are large and oval of optical units of ommatidium; B – thorax with anepisternum and mediotergite (below the scutellum) yellow with black spots are present on the margins of the thorax; C – wings with 4 yellow parallel crossband; one band occurs in region of sub basal, second one is on discal region, third originates on subapical and third and fourth band extends towards apical

The eggs were small, elongated spindle-shaped, and creamy white in colour (Figure 1A). The overall mean length and width of the egg was 0.61 mm and 0.18 mm, respectively (Table 1).

After hatching from the eggs, the maggots were tiny and could not be traced out in the pulp (Figure 1A). Hence, the observations on the description of the maggots and morphometric data were made at a three day interval after egg hatched.

The third day after the maggot hatched, it was creamy white in colour and started to feed on the pulp (Figure 1B). Its mean length varied from 4.81 mm, 4.84 mm and 4.77 mm, respectively, in Tamil Nadu, Punjab, and Rajasthan. The mean width of the maggots on the third day varied from 1.33 mm, 1.36 mm, and 1.41 mm, respectively, from the samples collected from Tamil Nadu, Punjab, and Rajasthan. The overall mean length and width of the maggots on the 3rd day after hatching from the egg were found to be 4.81 mm and 1.36 mm, respectively (Table 1).

The maggot on its fifth day after hatching looked creamy white in colour (Figure 1B). The anterior region was narrow, and the posterior region was found to be blunt. The maggot made a reddish-brown gal-

lery in the pulp while feeding. During that time, the mean length of the maggot measured 5.35 mm, 5.41 mm, and 5.51 mm, respectively, from Tamil Nadu, Punjab, and Rajasthan (Table 2). The mean width was 1.51 mm (Tamil Nadu), 1.52 mm (Punjab), and 1.54 mm (Rajasthan). The overall mean length and width of the maggot on the 5th day after hatching were recorded as 5.42 and 1.52 mm.

The maggots stopped feeding and body started to shrink. The maggot moved towards the skin of the fruit and made a circular hole in the skin to come out of the fruit for pupation. Very few maggots passed their pupal stage in the fruit itself. The maggots came out of the fruit from these circular holes and fell onto the ground. The full-grown maggot showed a peculiar habit of bringing together both ends of the body and then they jumped into the air and fell on the ground. The mean length of the maggot on its eighth day after hatching from an egg from Tamil Nadu, Punjab, and Rajasthan measured 6.18 mm, 6.27 mm, and 6.14 mm, respectively. The mean width was 1.88 mm (Tamil Nadu), 1.94 mm (Punjab), and 1.92 mm (Rajasthan). The overall mean length and width of the maggot



Figure 3. Morphological features of adult characters of ber fruit fly, *Carpomyia vesuviana* (Tephritidae; Diptera)
A – fore leg, mid leg and hind leg; B – abdomen with ovipositor; C – ovipositional punctures; D – adult male

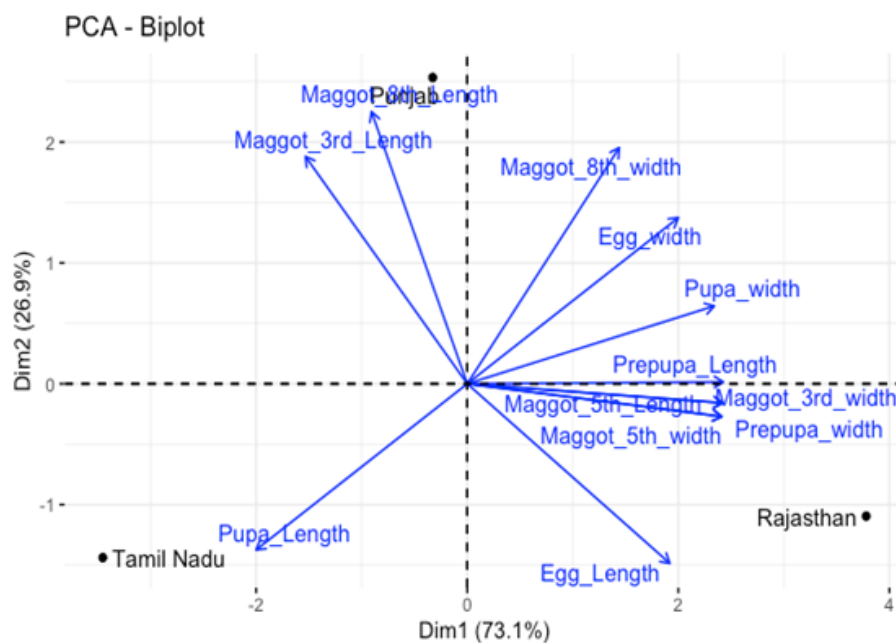


Figure 4. Biplot loading of the principal components showing relations among various Immature stages of *Carpomyia vesuviana*

Table 3. Morphometrics of *Carpomyia vesuviana* (male adult) from different locations in India

S. No.	Characters	Tamil Nadu		Punjab		Rajasthan		Overall Mean (mm)
		Range (mm)	Mean $\pm$ Std (mm)	Range (mm)	Mean $\pm$ Std (mm)	Range (mm)	Mean $\pm$ Std (mm)	
1		Antenna						
	Length	0.51–0.41	0.46 $\pm$ 0.02	0.49–0.44	0.48 $\pm$ 0.02	0.46–0.41	0.44 $\pm$ 0.01	0.46 $\pm$ 0.02
2		Head						
	Length	1.46–1.38	1.43 $\pm$ 0.03	1.41–1.38	1.39 $\pm$ 0.02	1.44–1.38	1.41 $\pm$ 0.02	1.41 $\pm$ 0.02
	Width	1.41–1.31	1.34 $\pm$ 0.03	1.36–1.24	1.27 $\pm$ 0.04	1.36–1.26	1.31 $\pm$ 0.03	1.31 $\pm$ 0.03
3		Thorax						
	Length	1.89–1.77	1.82 $\pm$ 0.04	1.79–1.75	1.78 $\pm$ 0.02	1.83–1.75	1.80 $\pm$ 0.03	1.80 $\pm$ 0.02
	Width	1.46–1.38	1.42 $\pm$ 0.03	1.44–1.31	1.37 $\pm$ 0.04	1.44–1.36	1.39 $\pm$ 0.02	1.39 $\pm$ 0.03
4		Abdomen						
	Length	0.98–0.81	0.90 $\pm$ 0.07	0.94–0.79	0.87 $\pm$ 0.05	0.98–0.75	0.85 $\pm$ 0.08	0.88 $\pm$ 0.03
	Width	0.85–0.71	0.77 $\pm$ 0.04	0.88–0.78	0.84 $\pm$ 0.03	0.84–0.66	0.77 $\pm$ 0.07	0.79 $\pm$ 0.04
5		Leg						
	Fore leg	1.18–1.01	1.11 $\pm$ 0.05	1.21–1.08	1.15 $\pm$ 0.04	1.22–1.05	1.19 $\pm$ 0.05	1.15 $\pm$ 0.04
	Mid leg	1.36–1.22	1.33 $\pm$ 0.04	1.39–1.31	1.35 $\pm$ 0.03	1.37–1.27	1.31 $\pm$ 0.03	1.33 $\pm$ 0.02
	Hind leg	1.77–1.39	1.56 $\pm$ 0.12	1.71–1.58	1.64 $\pm$ 0.05	1.69–1.46	1.58 $\pm$ 0.06	1.59 $\pm$ 0.04
6		Whole body						
	Length	4.24–4.11	4.18 $\pm$ 0.04	4.29–4.21	4.25 $\pm$ 0.03	4.21–4.05	4.14 $\pm$ 0.05	4.19 $\pm$ 0.05
	Wing expanse	3.83–3.59	3.69 $\pm$ 0.07	3.64–3.46	3.56 $\pm$ 0.06	3.53–3.33	3.43 $\pm$ 0.07	3.56 $\pm$ 0.13

$n = 10$ replications were taken with each location

on the 8th day after hatching measured 6.19 and 1.91 mm, respectively.

After falling on to the ground, the prepupa entered into the soil through wriggling movements of its body (Figure 1C). Up to 95% of the maggots pupated in the upper layer of the soil. Very few maggots did not fall on the ground and their prepupa and pupal stages took place in the fruits themselves. The mean length of prepupa was 7.52 mm, 7.66 mm, and 7.84 mm, respectively, in the Tamil Nadu, Punjab, and Rajasthan locations. The mean width of the prepupa from Tamil Nadu, Punjab, and Rajasthan varied from 1.62 mm, 1.63 mm, and 1.65 mm, respectively. The overall mean length and width of prepupa were 7.67 mm and 1.64 mm, respectively.

Initially, the pupa was creamy white and turned into a brown colour and had a barrel shape (Figure 1D). The mean length of the pupa from Tamil Nadu, Punjab, and Rajasthan was 4.17 mm, 3.92 mm, and 3.92 mm, respectively. The mean width of the pupa from Tamil Nadu, Punjab, and Rajasthan varied from 1.71 mm, 1.73 mm, and 1.74 mm, respectively. The overall mean length and width of the pupa were 4.00 mm and 1.72 mm, respectively (Table 1).

The adult is a small brownish-yellow fly with brown longitudinal stripes on the thorax. These stripes are surrounded by black spots on the sides of the margin. The females are slightly larger than the males. The mean length of whole female body was 4.73 mm, 4.75 mm, and 4.76 mm, respectively. The overall mean length of the female whole body and wing expanse measured 4.74 mm and 3.92 mm, respectively (Table 2). Concerning the whole male body length, it reached 4.18 mm, 4.25 mm, and 4.14 mm, respectively, as mentioned in Table 3 and Figure 3D. The mean width of the whole body of the females from Tamil Nadu, Punjab, and Rajasthan was 3.94 mm, 3.90 mm, and 3.93 mm, respectively, and the male whole width measured 3.69 mm, 3.56 mm, and 3.43 mm, respectively. The overall mean male whole body length and wing expanse was 4.19 mm and 3.56 mm, respectively (Table 3). In the thorax, in the Anepisternum and mediotergite (below the scutellum), yellow with black spots are present on the margins of the thorax (Figure 2B). The mean length and width of the female thorax from Tamil Nadu, Punjab, and Rajasthan measured 1.93 mm and 1.47 mm, 1.91 mm and 1.51 mm, 1.89 mm and 1.44 mm, respectively.

Table 4. Eigen analysis of correlation matrix in *Carpomyia vesuviana* immature stages of fruit flies

	PCA1	PCA2
Eigen values	8.772	3.228
Percentage of variance	73.102	26.898
Contribution features (variables)	Loadings	
Egg length	0.791	−0.612
Egg width	0.824	0.566
Length of maggot on 3 rd day after hatching	−0.632	0.775
Width of the maggot on 3 rd day after hatching	0.998	−0.065
Length of maggot on 5 th day after hatching	0.998	−0.065
Width of the maggot on 5 th day after hatching	0.994	−0.112
Length of maggot on 8 th day after hatching	−0.374	0.927
Width of the maggot on 8 th day after hatching	0.594	0.805
Prepupa length	1.000	0.006
Prepupa width	0.994	−0.112

as mentioned in Table 3, the length and width of the male thorax from Tamil Nadu, Punjab, and Rajasthan measured 1.82 mm and 1.42 mm, 1.78 mm and 1.44 mm, 1.80 mm and 1.39 mm, respectively (Table 3). The overall mean length and width of the female thorax measured 1.91 mm and 1.47 mm, respectively (Table 3), and the overall mean length

and width of the male thorax measured 1.80 mm and 1.39 mm, respectively (Table 3).

In the *C. vesuviana* wings, four yellow cross-bands are present. The first band occurs in the sub-basal region. The second one is in the discal region, and the third originates in the sub-apical region, where the third and fourth join together and extend to the apical margins of the wings (Figure 2C). The mean wing length of the females from Tamil Nadu, Punjab, and Rajasthan measured 3.87 mm, 3.93 mm, and 3.90 mm, respectively. The mean width of the female wings from Tamil Nadu, Punjab, and Rajasthan varied at 1.43 mm, 1.44 mm, and 1.46 mm, respectively, as shown in Table 3. The overall mean length and width of the female wings reached 3.90 mm and 1.44 mm, respectively. The mean length of the female antenna from Tamil Nadu, Punjab, and Rajasthan was 0.55 mm, 0.57 mm and 0.53 mm, respectively (Figure 2A). The overall mean length of the female antenna female was 0.55 mm. The mean length of the ovipositor was 0.33 mm, 0.34 mm, and 0.36 mm, from Tamil Nadu, Punjab, and Rajasthan, respectively (Figure 3B). The overall mean length of the ovipositor was 0.34 mm (Table 2). The mean length of the male abdomen was 0.90 mm, 0.87 mm, and 0.85 mm from Tamil Nadu, Punjab, and Rajasthan, respectively (Figure 3D). The mean width of the male abdomen from Tamil Nadu, Punjab, and Rajasthan measured 0.77 mm, 0.84 mm, and 0.77 mm, respectively. The overall mean length and width

Table 5. Eigen analysis of correlation matrix in *Carpomyia vesuviana* female adult characters

Characters	Female adult		Male adult	
	PCA1	PCA2	PCA1	PCA2
Eigen values	8.569	6.431	9.490	4.510
% of variance	57.125	42.875	67.784	32.216
Cumulative % of variance	57.125	100.000	67.784	100.000
Contribution features (variables)	Loadings			
Antenna length	0.702	0.298	−0.723	0.690
Head length	0.801	0.199	0.960	0.281
Head width	0.702	0.298	0.980	0.201
Thorax length	0.003	0.997	0.960	0.281
Thorax width	0.774	0.226	0.921	0.389
Fore leg length	0.857	0.143	−0.236	−0.972
Mid leg length	0.924	0.076	−0.723	0.690
Hind leg length	0.796	0.204	−1.000	−0.004
Wing length	0.796	0.204	0.999	0.043
Wing width	0.018	0.982	0.972	−0.236

of the male abdomen was 0.88 mm and 0.79 mm, respectively as shown in Table 3. The mean length of the female abdomen from Tamil Nadu, Punjab, and Rajasthan measured 1.85 mm, 1.88 mm, and 1.88 mm, respectively, then the mean width of the female abdomen measured 1.62 mm, 1.69 mm, and 1.65 mm, respectively. The overall mean length and width of the female abdomen measured as 1.87 mm and 1.65 mm, respectively, as mentioned in Table 3. The overall mean length of the female fore leg, mid leg and hind leg from Tamil Nadu, Punjab, and Rajasthan measured 1.13 mm, 1.26 mm, and 1.53 mm (Table 3 and Figure 3A), respectively. Then, the overall mean length of the male fore leg, mid leg and hind leg from Tamil Nadu, Punjab, and Rajasthan measures 1.15 mm, 1.33 mm and 1.59 mm (Table 3 and Figure 3A), respectively.

Results of the PCA analysis for the immature stages. The PCA results revealed that PCA1 accounted for 73.10% of the total variance, while PCA2 explained 26.90% (Table 4). The key contributors to PCA1 included the length and width of the maggots on the 8th day after hatching, the pupa length, and egg width, with the arrows pointing to the right, indicating their significant influence. Rajasthan's proximity to these variables suggests a strong correlation between these traits and the region. In contrast, the pupa and egg lengths were inversely related to the maggot length on the 3rd day, as reflected by their opposing arrow directions. Tamil Nadu's position on the left side of the plot indicates higher values for these variables in the samples from the region (Figure 4).

In PCA1, the prepupa length (1.000), maggot width on the 3rd day and the maggot length on the 5th day (0.998) showed the highest positive loadings, indicating a strong association with PCA1 (Table 5). The width of the maggot on the 5th day (0.994) and the maggot length on the 3rd day (−0.632) followed. In PCA2, the maggot length and width on the 8th day (0.927 and 0.805) displayed prominent positive loadings (Table 6).

Results of the PCA analysis for the female JFF. The PCA results indicated that PCA1 explained 57.12% of the variance with an eigenvalue of 8.569, while PCA2 accounted for 42.87% of the variance with an eigenvalue of 6.431 (Table 7). The antenna length, head length, and head width showed strong associations with PCA1, contributing 70.2%, 80.1%, and 70.2%, respectively. The thorax length had a near-perfect alignment with PCA2, contributing 99.7%, while the thorax width was primarily linked to PCA1 (77.4%) with the remainder explained by PCA2. The mid leg length, with the highest contribution (92.4%), and fore leg length, hind leg length, and wing length all had significant associations with PCA1. The wing width, however, was more strongly associated with PCA2 (98.2%) (Table 7).

The PCA biplot showed that Punjab was closely aligned with variables like the head length, abdomen length, and wing length, while Tamil Nadu showed lower values for these traits. Rajasthan, on the other hand, was closely associated with the fore leg length and wing expanse, highlighting regional differences in these characteristics.

Results for the PCA analysis for the male fruit flies. The PCA results showed that PCA1 had

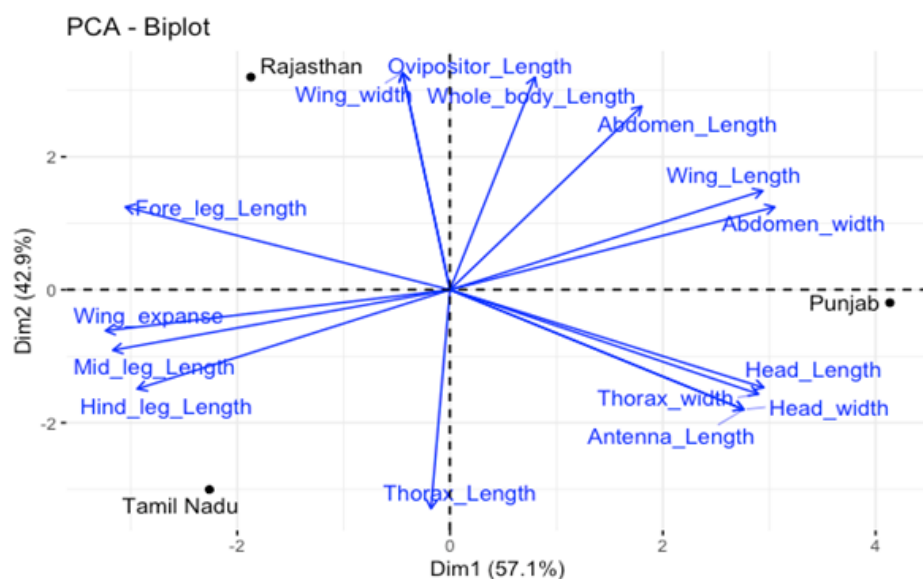


Figure 5. Biplot loading of the principal components showing relations among various female adult stages of *Carposymia vesuviana*

Table 6. Sequence analysis results by using BLAST confirmation with US NCBI under similar accession number

Name of species isolates	Strains	Accession number	Accession length (bp)	Query cover (%)	Expected values	Percent identify (%)	Accession length (bp)	Similarity NCBI accession number
<i>Carpomyia vesuviana</i> Costa	CV1	PQ198003	638	100	0.0	99.26	16 083	NC_071721.1 (Beijing, China)
		Tamil Nadu		100	0.0	99.26	16 076	MT121231.1 (Beijing, China)
	CV2	PQ198005	683	100	0.0	99.56	16 083	NC_071721.1 (Beijing, China)
		Punjab		100	0.0	99.56	16 076	MT121231.1 (Beijing, China)

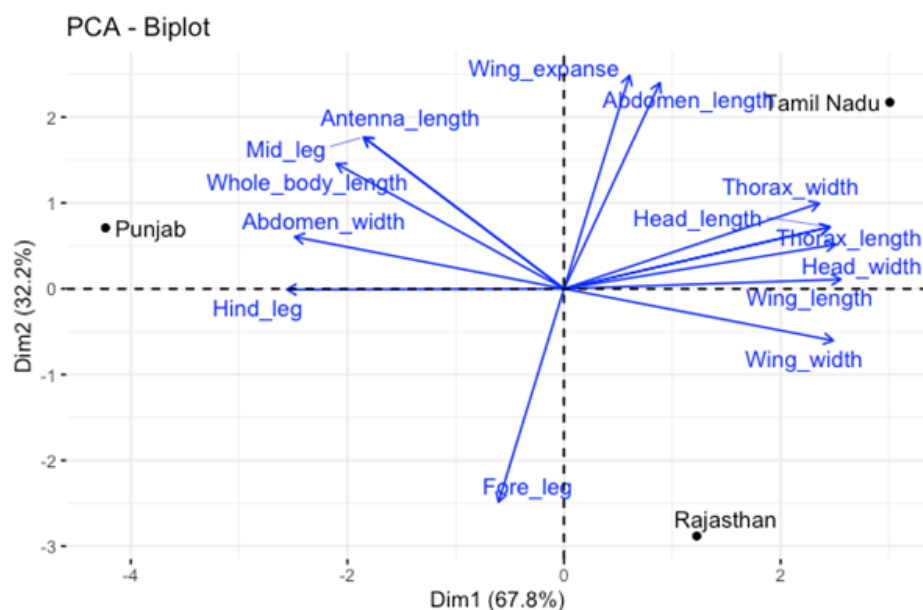
NCBI – National Center for Biotechnology Information

a variance of 9.49, accounting for 67.78% of the total variance, while PCA2 had a variance of 4.51, contributing 32.21% (Table 7). Specimens from Rajasthan and Tamil Nadu were strongly associated with positive Dim1 values, indicating that traits like the wing expanse, abdomen length, thorax dimensions, and head measurements were key contributors to this variation. Rajasthan showed a strong correlation with these traits. In contrast, specimens from Punjab were positioned on the negative side of Dim1 and the positive side of Dim2 (Figure 6), suggesting that traits, such as the hind leg length, abdomen width, whole body length, mid leg, and antenna length, were more prominent in Punjab, distinguishing it morphologically from the other regions.

PCA1 revealed strong positive loadings for the head length (0.960), head width (0.980), thorax length (0.960), thorax width (0.921), and wing length (0.999), indicating their major contribution to this component. In contrast, the antenna length (−0.723), mid leg length (−0.723), and hind leg length (−1.000)

showed strong negative loadings, suggesting an inverse relationship with these features. PCA2, on the other hand, identified different associations. The antenna length (0.690), head length (0.281), head width (0.201), thorax length (0.281), and thorax width (0.389) made modest positive contributions, while the fore leg length (−0.972) and hind leg length (−0.004) displayed strong negative loadings, highlighting their distinct role in PCA2.

Molecular analysis. The results of the PCR product showed two clear bands, plus a ladder, with a size of about 638 base pairs, confirming the amplification of the COXI–COXII target region. The molecular analysis based on the PCR and sequencing of the COXI–COXII region confirmed the species identity. The nucleotide sequence of *C. vesuviana* from Tamil Nadu and Punjab was deposited in GenBank as PQ198003 and PQ198005 which had nearly 99% genetic similarity with two sequences NC_071721.1 (Beijing, China) and MT121231.1 (Beijing, China) submitted in the NCBI database (Table 4).

Figure 6. Biplot loading of the principal components showing relations among various male adult stages of *Carpomyia vesuviana*

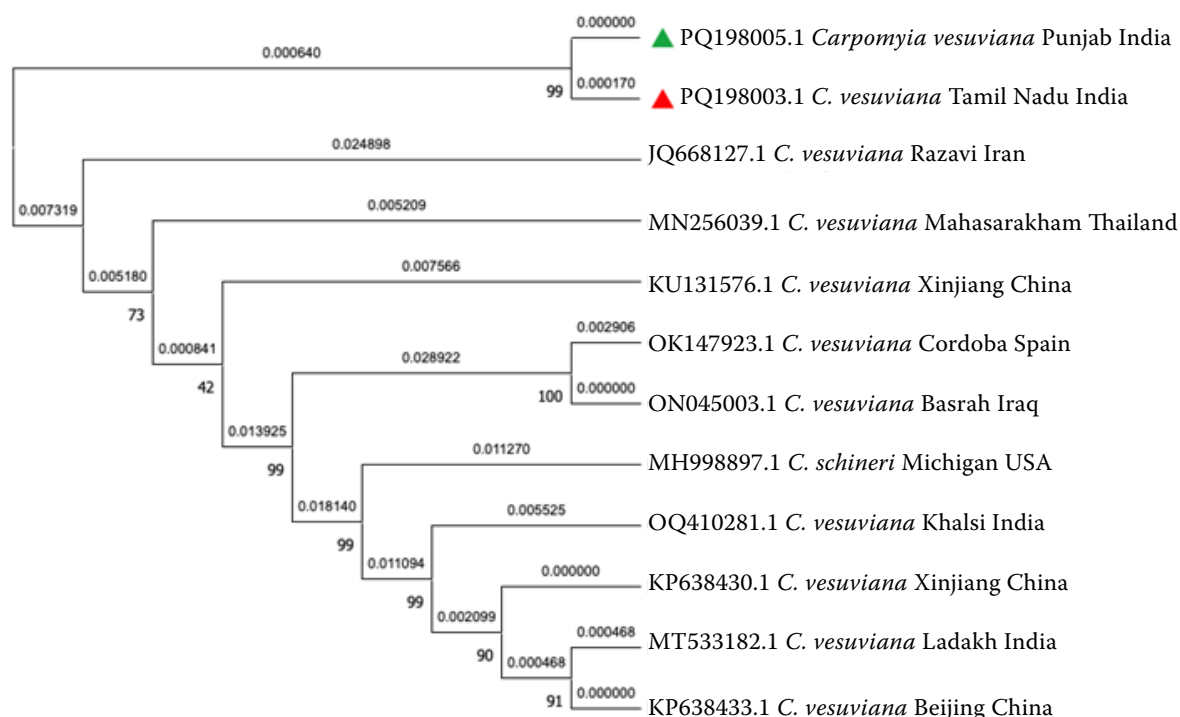


Figure 7. Phylogenetic tree showing its genetic relationships with other *Carpomyia* spp.

Phylogenetic analysis. The phylogenetic analysis showed distinct clustering of samples, highlighting regional differences. The tree included samples from India (Tamil Nadu, Punjab, Khalsi, Ladakh), Iran (Razavi), Thailand (Mahasarakham), China (Xinjiang, Beijing), Spain (Cordoba), Iraq (Basrah), and the USA (Michigan). Bootstrap values and branch lengths were used to support the genetic relationships, as shown in (Figure 7).

The Tamil Nadu (PQ198003.1) and Punjab (PQ198005.1) samples exhibited minimal genetic divergence (0.000000–0.000170), indicating strong genetic similarity. These samples formed a distinct cluster with high bootstrap support (99), confirming the robustness of this grouping. This suggests a close genetic relationship between the species in Tamil Nadu and Punjab, which was also closely related to a sample from Razavi, Iran (JQ688127.1).

The branch length between the Punjab/Tamil Nadu cluster and the Iran sample was slightly larger (0.024898). It indicates more genetic divergence between the Indian and Iranian populations. The sequence of the *C. schineri*, Michigan, USA sample was the most genetically divergent, positioned on a branch with a bootstrap value of 100 and a branch length of 0.019492.

From the tree, samples of *C. incompleta* (ON045003.1, Basrah, Iraq) and *C. schineri*

(MH998897.1, Michigan, USA) form a separate branch from all the other samples. These taxa had the longest branch lengths and the highest divergence compared to the other samples in the tree. Their distant relationship suggests they are evolutionarily distinct from the other samples, which may all belong to a different species or closely related group.

The *C. vesuviana* sample from Khalsi, India, formed a distinct branch with a bootstrap value of 90 and a branch length of 0.005655. The Xinjiang and Beijing samples from China clustered closely, showing a high bootstrap value of 99 and a short branch length of 0.000589, indicating genetic similarity likely due to the geographical proximity. The sample from Cordoba, Spain, was more closely aligned with the Basrah, Iraq sample, with a bootstrap value of 91 and a branch length of 0.019250, possibly reflecting historical migration or trade routes.

DISCUSSION

Jujube fruits can host *C. vesuviana* as well as *C. incompleta* Becker, 1903. Therefore, it was decided to find out which of the two species was responsible for the damage observed in various parts of India. Based on the taxonomic identification

using taxonomic keys, the adults were identified as *C. vesuviana*.

Concerning the morphometric analysis, it was observed that the mean length and width of the egg varied from 0.58 mm and 0.16 mm, 0.54 mm and 0.19 mm, 0.72 mm and 0.19 mm, respectively. However, the overall mean length and width of the egg were 0.61 mm and 0.18 mm, respectively. Similar findings were reported by Joshi and Sinde (1971) who reported that, on average, the egg measured 0.88 mm in length and 0.23 mm in width. In the study conducted by Kavitha and Savithri (2002), they reported that the length and width of the egg were 0.60 mm and 0.16 mm, respectively. However, Haldhar (2021) reported that the mean length and width of the egg were 0.86 mm and 0.20 mm, respectively.

In this present study, the full-grown maggot length and width varied from 6.18 mm and 1.88 mm, 6.27 mm, and 1.94 mm and 6.14 mm and 1.92 depending on the locality (Table 1). According to Haldhar (2021), the length and width of maggot measured 7.72 mm and 1.50 mm, respectively. Kavitha and Savithri (2002) reported that the length and width of full-grown maggot were 7.74 mm and 1.50 mm, respectively.

According to (Haldhar 2021), the length and width of the prepupa were measured as 7.59 mm and 1.67 mm, respectively. Kavitha and Savithri (2002) also reported that the length and width of the prepupa were 7.56 mm and 1.60 mm, respectively. These findings support our current results, in which the average length and width of prepupa were recorded as 7.52 mm and 1.62 mm, 7.66 mm and 1.63 mm, and 7.84 mm and 1.65 mm, respectively.

The findings of Kavitha and Savithri (2002) revealed that the length and width of the pupa were 4.26 mm and 1.90 mm. However, Haldhar (2021), reported that mean length and width of the pupa measured 4.02 mm and 1.69 mm.

This study revealed that the mean length of the female whole body was 4.73 mm, 4.75 mm, and 4.76 mm, respectively. With respect to male whole body length, it was 4.18 mm, 4.25 mm, and 4.14 mm, respectively. Similar results were reported by the following authors: Kavitharaghavan et al. (2005) stated that the length and width of male adults measured 4.65 mm and 1.29 mm, respectively. The length and width of the female adults was 4.38 mm and 1.15 mm, respectively. The overall mean length of the female and wing expanse was 4.74 mm and 3.92 mm, respectively (Table 3).

According to Haldhar (2021), the male adult of JFF measured 4.44 mm in length and 1.21 mm in width. The mean length of the wing expanse of the male adults was 3.83 mm. He also observed that the length and width of the female JFF measured as 4.82 mm and 1.33 mm, respectively. He also reported that the wings expanse of female adults was 3.91 mm. The morphological markers of the adult jujube fruit fly are congruent with the findings of Pollini and Cravedi (2014). Limited studies had been conducted on *C. vesuviana* morphometrics. Based on the morphometrics measurements, the current specimen was clearly identified and differentiated from *C. incompleta*.

The study conducted by Taher and Alyousuf (2023) on the morphometrics of *C. incompleta* revealed that the adult was characterised by its small size, yellow colour, streamlined compressed shape, and a length of 4.91 mm and 4.11 mm for females and males, respectively. The shortest distance between the two ends of the extended wings was recorded as 7.13 mm, while the ovipositor measured approximately 0.63 mm in length and 0.13 mm in width. The thorax region of *C. incompleta* showed three lines of dark hairs on the dorsal surface of the scutellum; with two dark spots on the lower side of the scutellum. The forewing was distinguished by the presence of three pale cross band, the first band appeared in the sub-basal region second band occurred in the discal region, and the third band was seen in the subapical region, but all the bands remained faint.

The highest positive loadings were observed. Based on the PCA biplot results of adult female JFF, it was evident that head length, abdomen length, and wing length were the major contributing factors in Punjab, whereas in Rajasthan, fore leg length and wing expanse played a significant role.

The head length, head width, thorax length, thorax width, and wing length contributed significantly to the adult female JFF. This is the first attempt of a morphometric analysis and PCA for JFF from various parts of India. Hence, a limited number of studies were available pertaining to this work.

Studies on the molecular characterisation of *C. vesuviana* are limited, prompting the present investigation to fill this gap. In this research, the key morphological markers used for species identification were subsequently validated through DNA barcoding. DNA barcoding is a fast and reliable method for distinguishing species (Menabit et

al. 2022), making it a valuable tool for confirming morphological identifications. In this study, the mitochondrial COXI–COXII marker was successfully used to identify the species by using the adult stage as *C. Vesuviana*.

In our present study, the samples of *C. incompleta*, *C. schineri* and *C. vesuviana* were closely related with each other. These taxa exhibited the longest branch lengths, and the highest divergence compared to other samples in the phylogenetic tree. Their distant relationship suggests they are evolutionarily distinct from the other samples, which may belong to a different species or a closely related group.

Interestingly, a phylogenetic analysis based on morphological traits conducted by Norrbom (1997) to determine the relationships among *Carpomyia* species revealed an almost identical clustering pattern as shown in Figure 7. In this analysis, *C. vesuviana* was closely related to *C. incompleta* with both species belonging closely aligned with *C. schineri*.

A similar study conducted by Taher and Alyousuf (2023) further supported this relationship demonstrating that bootstrap values indicated a closer genetic relationship between *C. incompleta* and *C. vesuviana* particularly with the sequences MT533182.1 from Ladakh and KP638433.1 from China. Their phylogenetic analysis divided the species into two major clades. The first clade comprised two closely related clusters: *C. vesuviana* and *C. Incompleta*, while the second clade was represented by *C. schineri*. This highlights the importance of integrating morphological and molecular data to ensure the accurate identification of insect species, especially those that are closely related leading to a more precise classification of the examined samples.

This article provides a comprehensive pictorial guide, supported by taxonomic characteristics, for the accurate identification of *C. vesuviana* in ber (jube). The rapid confirmation of the species identity is further validated through molecular characterisation, ensuring precise identification for effective management solutions. Infestation of ber fruits often occurs alongside other fruit fly species, including *Bactrocera correcta* (Bezzi), *B. dorsalis* (Hendel), and *B. zonata* (Saunders), highlighting the need for the accurate differentiation of species to implement targeted control measures. However, managing fruit flies in the *ber* ecosystem remains challenging and requires a multifaceted approach to achieve long-term success.

Currently, Integrated Pest Management (IPM) modules are available only for *B. dorsalis*, *B. correcta*, and *B. zonata* in jube cultivation. These modules include a combination of cultural, mechanical, and monitoring practices, such as raking the soil around trees to expose pupae to heat and natural enemies, the periodic collection and deep burial (60 cm) of infested fruits to prevent re-emergence, shallow ploughing (4–6 cm depth) with a cultivator to expose and eliminate pupating larvae and pupae, maintaining weed-free fields to minimise alternate host plants and deploying fruit fly traps (16 traps/acre) at monthly intervals for monitoring and control (Sharma & Sandeep 2011).

Despite these efforts, *C. vesuviana* presents a unique challenge as it is not attracted to conventional lures, such as methyl eugenol (ME), a cue lure, and other pheromone-based traps commonly used for *Bactrocera* species. Therefore, identifying a suitable attractant for *C. vesuviana* is critical for developing species-specific monitoring and management strategies. Electroantennogram (EAG) studies hold great potential in providing valuable insights into the olfactory receptors of *C. vesuviana*, facilitating the identification of key chemical cues involved in host seeking behaviour.

The future of fruit fly management in the *ber* ecosystem appears promising through the integration of innovative techniques and research-driven approaches. Special emphasis on biocontrol strategies is essential for sustainable, long-term pest management strategies. Identifying and promoting potential natural enemies can play a pivotal role in maintaining an ecological balance and reducing fruit fly populations effectively. Moving forward, an increased focus on chemical ecology, molecular tools, and biological control agents will be key to overcoming the challenges posed by *C. vesuviana* and other fruit fly species in the *ber* ecosystem.

CONCLUSION

The study provided a comprehensive insight into the morphological, morphometric, and molecular characteristics of *C. vesuviana* across different geographic locations in India, including Tamil Nadu, Punjab, and Rajasthan. The adults fruit flies infesting jube fruit were identified taxonomically as *C. vesuviana* Costa. The morphometric analysis

of the species, from the immature stages to adulthood, showed clear differences between the regions, with variations in traits such as the egg, maggot, prepupa, pupa, and adult measurements

A molecular analysis using the COXI–COXII gene region confirmed the species identity as *C. vesuviana*, with samples from Tamil Nadu and Punjab showing a high degree of genetic similarity, as indicated by the phylogenetic tree. Both the morphometric and molecular characterisation provide complementary insights into the insect biology. Together, they form a powerful toolkit for advancing agricultural sustainability, biodiversity conservation, and ecosystem management strategies.

Acknowledgments

The authors thank Dr. R.K. Meena for the samples from Rajasthan and all the authors thank Dr. P.D. Kamala Jayanthi, ICAR-National Professor, ICAR-IIHR, Bengaluru, India who facilitated Dr. Arul Dhayalan, Dr. M. Sumathi and Dr. Radhika Hedge from ICAR-IIHR, Bengaluru, India for conducting the molecular sequencing and Dr. K.J. David, Principal Entomologist, ICAR-NBAIR, Bengaluru for confirming the morphological identification. Last, but not least, my sincere gratitude to Dr. Gandhi Gracy (Principal Scientist) and Dr. Selva Babu (Entomologist) from ICAR-NBAIR, Bengaluru, India, who helped to assemble the sequences for submission at NCBI, USA.

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Received: September 17, 2024

Accepted: April 2, 2025

Published online: October 8, 2025