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A comparative study of morphological characterization and developmental biology of *Maruca vitrata* (Fabricius, 1787) populations across India

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Abstract

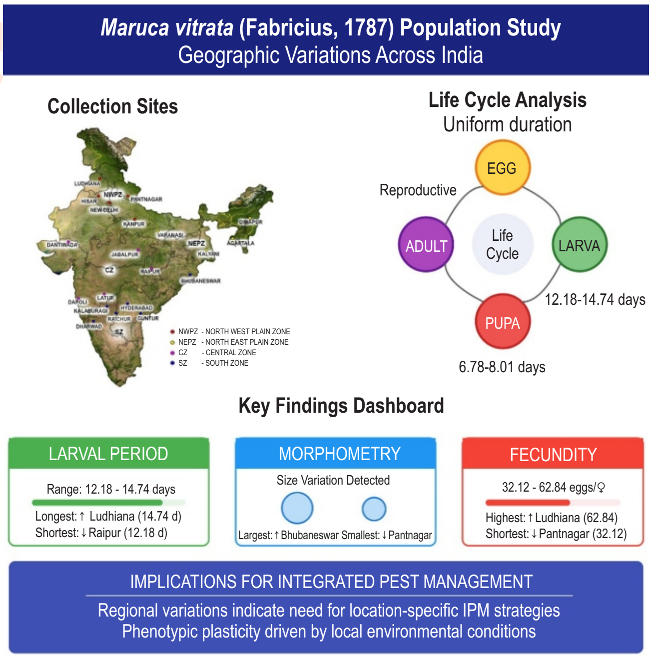
Aim: The legume pod borer, *Maruca vitrata* (Fabricius, 1787), is an agriculturally important and major economic insect-pest of leguminous crops cultivated in tropical and subtropical regions. This study aimed to analyse the morphological and biological variations in different *M. vitrata* populations collected from eight legume-growing locations in India.

Methodology: The morphometric characteristics and various developmental stages of *M. vitrata* populations collected from eight locations of India were studied. Under controlled laboratory conditions, the immature stages of eight populations were reared until maturity. Subsequently, the data on life cycle duration, morphometric observations of different larval instars, fecundity, and sex ratio were collected and growth indices were analyzed.

Results: Significant geographic variation was observed among the eight *M. vitrata* populations, with clear differences in developmental durations, and total life cycle. Morphometric comparisons revealed consistent regional differences in body dimensions, while larval chaetotaxy remained conserved.

Interpretation: This study provide a crucial understanding of its biology, as well as its morphologically important phenotypic traits. These comparative data on detailed bionomic and morphological aspects may aid in the formulation of effective and phenology-based integrated pest management strategies for this economically important lepidopteran insect pest of legume.

Key words: Developmental biology, Geographical variation, Legume pod borer, *Maruca vitrata,* Morphometric analysis, Pigeonpea



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Introduction

The spotted pod borer, *Maruca vitrata* (Fabricius, 1787) (Lepidoptera: Crambidae) is an agriculturally important pest of leguminous crops in tropical and subtropical countries (Singh and Emden, 1979), including Asia and Africa (Agunbiade *et al.,* 2014). It affects more than 39 host plant species (Ganapathy, 2010), as the larvae develop within the webbed clusters of leaf axils, flowers and pods which serve as a protection against the natural enemies and reduce insecticide efficacy (Mahalle and Taggar, 2018). In India, *M. vitrata* has become a serious pest of early- and medium-late-maturing pigeonpea production with the indiscriminate use of insecticides resulting in this kind of development of insecticide resistance (Sahoo, 1995; Rachappa *et al.,* 2017; Sreekanth *et al.,* 2015). The current findings support the advancements in developing a newer and more sustainable Integrated Pest Management (IPM) strategies that can mitigate the adverse effects of this pest on legume crop yields. Proper taxonomic identification of species within the genus *Maruca* Walker, F., 1859 is challenging, suggesting a species complex (Mahalle *et al.,* 2022; Taylor, 1967). Through molecular studies, Margam *et al.* (2011) revealed different genetic lineages in Asian and African populations, thus, confirming the possibility of a species complex in *M. vitrata*. Intraspecific morphological variation can be affected by abiotic factors (temperature, humidity) or biotic variables (host availability) as in other pest species such as *Spodoptera frugiperda* (Bale *et al.,* 2002).

Such variation underscores the need for conducting additional research on local insect populations. Morphometry is the statistical calculation of the biological measurements of size, shape, determining instar, etc., of a living or dead organism which estimate the genetic and phenotypic variations (Benson *et al.,* 1982). In Entomology, the wings, legs, etc. are the measure for deciphering the connections to life history, genetics, ecology, and systematics (Daly, 1985). It provides an unbiased, faster and cost-effective alternative for supplementing traditional taxonomy and molecular research (Daly, 1985; Smith-Pardo *et al.,* 2025). It an effective tool for differentiating morphologically identical insect species (Sheoran *et al.,* 2023). Geographic sampling at different environments can allow measuring morphological diversity and its relationship to morpho-functional mechanisms (Gigena *et al.,* 2023). Despite various studies conducted on *M. vitrata* in India, comparative studies where morphological and biometric variation has been studied with geographical locations are lacking. This study aims at uncovering the morphological distinctions in the populations of *M. vitrata* from eight locations in India under controlled laboratory conditions in order to contribute to basic knowledge to formulate pest management strategies for the region.

Materials and Methods

Field sampling and identification: *M. vitrata* larvae were collected from pigeonpea sites in India. About 150-200 larvae were randomly collected at each site. The experiments were conducted at Banaras Hindu University, India. The larvae were identified using a setal map and diagnostic key by Gilligan and Passoa (2014). Morphological descriptions and developmental periods were documented in populations collected from the second generation.

Laboratory rearing and biology study: Larvae were reared in an insect-rearing chamber (Instech Environmental Chamber, Instech Systems, India) under controlled laboratory conditions (26±1 °C temperature, 60±10% relative humidity (% RH), and a photoperiod of 14:10 hr light/dark). *M. vitrata* larvae were first reared on their host plant (pigeonpea) to complete the first generation. Thereafter, these were subjected to laboratory culture. Newly hatched second-generation larvae were raised on a chickpea-pigeonpea-based semi-synthetic diet (Mahalle and Srivastava, 2020). Biological parameters including the developmental periods of immature stages (egg, larva, pre-pupa, and pupa), adult longevity, total lifespan, pre-oviposition, oviposition, and post-oviposition periods, sex ratio, and fecundity were recorded from five completely randomized replicates of 50 larvae. The larval growth index (LGI), standardized insect-growth index (SII) and fitness index (FI) of the study populations were calculated by the formula of Amer and El-Sayed (2014).

Morphometric studies of different stages of *M. vitrata*: Morphometric measurements were taken from 50 randomly selected specimens from each developmental stage (eggs, larvae, head capsules, and pupae) of the insect cultures at each location. The length and maximum body width were measured with a stereoscopic binocular microscope with an image analyzer (Leica™) and expressed in millimeters (mm). Pupal weight was recorded using a microbalance and expressed in milligrams (mg). Morphometric traits of 50 adult moths (both sexes) were recorded.

Statistical analyses: JMP Statistical Discovery software (version 5.2, SAS Institute 2013) was used to analyze all the biometric data, which was presented as the mean±standard deviation (SD). One-way ANOVA was used to analyse the experimental data, and the significant variations between the mean values ($p \leq 0.05$) were calculated by Tukey test.

Results and Discussion

The present study demonstrated significant geographical variation in the biology and development of *M. vitrata* in different geographical regions of India. Observations were conducted on all important life stages (eggs, larvae, pupae, adults) and measures of growth, longevity, reproduction and population fitness. Incubation of eggs ranged from 2 to 3 days among the populations and was 2.66±0.12 days in Raipur to 2.92±0.09 days in Ludhiana (Table 2). The longest incubation period was observed in Ludhiana but was similar to most other populations except Bhubaneswar and Raipur. These findings are in accordance with the previous reports, which reported incubation periods of 2.80 days (Naveen *et al.,* 2009), 2.50 days (Chaitanya *et al.,* 2012), 3.65 days (Sravani and Mahalakshmi, 2016), 2.85 days (Rachappa *et al.,* 2017), 2.63 days (Savde *et al.,* 2018), and

Table 1: Sampling details of the *M. vitrata* populations collected from different locations across India

Sampling location	Altitude (msl)	Agroclimatic zone	State	Latitude (°N)	Longitude (°E)	Sampling date
Bhubaneswar	43 m	East Coast Plains and Hills Region	Orissa	20.265	85.8115	December, 2019
Raipur	317.3 m	Chhattisgarh Plains	Chhattisgarh	21.2382	81.7048	December, 2019
Varanasi	82.3 m	Middle Gangetic Plains Region	Uttar Pradesh	25.2677	82.9913	November, 2019
Kanpur	126-127 m	Upper Gangetic Plains Region	Uttar Pradesh	26.44	80.33	October, 2019
New Delhi	216-218 m	Trans-Gangetic Plains Region	Delhi	28.6377	77.1571	October, 2018
Pantnagar	243.84 m	Tarai and Hilly Areas	Uttarakhand	29.0222	79.4908	October, 2018
Hisar	212-213 m	Trans-Gangetic Plains Region	Haryana	29.1416	75.7112	October, 2018
Ludhiana	254.2 m	Agroclimatic Zone III	Punjab	30.901	75.8071	October, 2018

2.47 days (Mahankuda and Tiwari, 2020). Recently, Sambathkumar *et al.* (2023) recorded an incubation period of *M. vitrata* of 2.83 days, depending on the host plants.

A significant difference in total larval periods was recorded at 12.18±0.38 and 14.74±0.42 days in Raipur and Ludhiana, respectively (Table 2). The duration of the individual larval instars was also different, and may have been attributed to environmental or host plant differences. The range of the larval period varied between 12.18-14.74 days among the populations (Table 2). These findings corroborate with the previous studies reporting cumulative larval durations of 14.85 days (Sravani and Mahalakshmi, 2016), 14.28-14.78 days (Rachappa *et al.,* 2017), 9.61 days (Savde *et al.,* 2018), 12.53 days (Mahankuda and Tiwari, 2020), 8.0-16.3 days (Sambathkumar *et al.,* 2023), and 13.24 days (Patel *et al.,* 2023). The pre-pupal stage took 2.08-2.56 days, while the pupal period ranged from 6.78-8.01 days. Particularly, Ludhiana showed the longest pre-pupal and pupal developmental period (2.56±0.10 and 8.01±0.13 days, while Raipur showed the least (2.08±0.11 and 6.78±0.12 days (Table 2). These results are congruous with earlier reports of pre-pupal and pupal durations between 1.43-2.40 days and 3.31-7.95 days, respectively (Mahankuda and Tiwari, 2020; Patel *et al.,* 2023; Rachappa *et al.,* 2017; Savde *et al.,* 2018; Shejulpatil *et al.,* 2020; Sravani and Mahalakshmi, 2016).

Significant differences in adult longevity were found between sexes and across populations. Generally, females live longer than males in Lepidoptera, is consistent with the previous reports. Ludhiana population recorded the longest adult lifespans for both males (7.90±0.18 days) and females (9.64±0.15 days), while Bhubaneswar had the shortest (males: 5.16±0.24 days; females: 7.26±0.12 days) (Table 2). The results of this study, showed that, in general, female *M. vitrata* live longer than the male, which confirms the previous findings. Similar findings were reported by Sambathkumar *et al.* (2023), who found that the average female lifetime (3.64 days) was greater than males (3.15 days). Rachappa *et al.* (2017) reported that the male and female longevities of 7.50-11.25 and 9.25-11.50 days, respectively. Extended female life spans may be due to reproductive biology enabling extended oviposition periods. The complete life cycle of *M. vitrata* significantly varied across geographical locations, with average 29.16- 36.13 days for males and 30.96-37.87 days for females (Table 2). Ludhiana recorded the longest, and Raipur the shortest durations of both males and females lifespan (Table 2). These values fall corroborates with the previous report duration of 32.95 to 35.10 days was recorded in Guntur (Sravani and Mahalakshmi, 2016), 36.21 to 37.87 days in Kalaburagi (Rachappa *et al.,* 2017), 30.62 to 34.59 days in Rahuri (Shejulpatil *et al.,* 2020), 28 to 33.50 days in Pantnagar (Mahankuda and Tiwari, 2020), and 29.82 to 32.36 days in Anand (Patel *et al.,* 2023). However, recently a shorter mean lifecycle period of 20.58 days has been recorded for various hosts in Coimbatore (Sambathkumar *et al.,* 2023).

Notable differences in growth and fitness indices were also observed among the populations (Table 2). The Raipur population exhibited the highest larval growth index (LGI) (6.72±0.32), indicating faster larval development. In contrast, the Pantnagar recorded the lowest LGI (5.34±0.17) (Table 2). In addition, Bhubaneswar population also had a much higher standardized insect growth index (SIGI) and fitness index (FI) (4.13±0.18 mg per day and 2.14±0.10 mg per day, while Pantnagar population had the lowest SIGI and FI (2.93±0.15 and 1.38±0.07 mg per day (Table 2). Panickar and Jhala (2007) earlier reported growth indices of 5.71 and 2.82 for cowpea and pigeonpea, respectively, which was less than that in the present study. Sambathkumar *et al.* (2023) reported the growth index ranging between 5.09 to 7.45; whereas, Patel *et al.* (2023) recorded the growth index of 7.55.

The study showed significant differences in pre-oviposition (2.10-2.76 days), oviposition (3.04-3.98 days), and post-oviposition (2.12-2.90 days) periods of the female *M. vitrata* moths in different geographic locations (Table 3). Ludhiana had the longest duration while Raipur had the shortest. These results coincide with the findings of Sambathkumar *et al.* (2023), who reported variations in pre-oviposition (1.2 to 2.2 days) and oviposition (1.8 to 3.4 days) across various hosts of *M. vitrata* (Table 3). Rachappa *et al.* (2017) recorded the pre-oviposition, oviposition, and post-oviposition periods (3.50, 4.50, and 2.25 days) on an artificial diet in Guntur, while Shejulpatil *et al.* (2020) further reported longer durations (4.37, 2.00, and 4.62 days) on pigeonpea at Rahuri. The current study also revealed variability in both fecundity and sex ratios of *M. vitrata* populations across India. Ludhiana had the highest fecundity (62.84 eggs per female)

Table 2: Developmental period of various life stages and relative growth and fitness indices of *M. vitrata* populations collected from different locations across India

Location	Developmental period (days)								Growth and Fitness Indices		
	Incubation period	Total larval period	Pre-pupal period	Pupal period	Adult longevity		Total life cycle				
					Male	Female	Male	Female	LGI	SIGI	FI
Ludhiana	2.92±0.09[a]	14.74±0.42[a]	2.56±0.10[a]	8.01±0.13[a]	7.90±0.18[a]	9.64±0.15[a]	36.13±1.09[a]	37.87±1.15[a]	6.44±0.26[ab]	3.22±0.10[bc]	1.97±0.10[ab]
Hisar	2.88±0.10[ab]	14.31±0.37[ab]	2.48±0.07[ab]	7.85±0.12[a]	7.58±0.19[a]	9.30±0.18[a]	35.10±1.17[a]	36.82±1.24[a]	5.92±0.25[bc]	3.21±0.16[bc]	1.76±0.12[bc]
New Delhi	2.85±0.07[abc]	14.04±0.46[abc]	2.40±0.07[abc]	7.51±0.11[b]	7.14±0.24[b]	9.24±0.14[a]	33.91±1.13[ab]	36.01±1.27[ab]	5.88±0.24[bcd]	3.21±0.17[bc]	1.72±0.09[cd]
Pantnagar	2.82±0.06[abc]	13.60±0.31[bcd]	2.32±0.09[bcd]	7.30±0.15[bc]	5.78±0.14[de]	7.94±0.20[c]	31.82±1.10[bc]	33.98±1.23[bc]	5.34±0.17[d]	2.93±0.15[c]	1.38±0.07[e]
Kanpur	2.77±0.10[abc]	13.25±0.25[cd]	2.25±0.08[cde]	7.18±0.09[c]	6.50±0.27[c]	8.62±0.23[b]	32.00±1.11[bc]	34.07±1.10[bc]	5.66±0.24[d]	3.14±0.14[bc]	1.52±0.09[de]
Varanasi	2.74±0.08[abc]	12.89±0.54[de]	2.18±0.12[de]	7.05±0.10[cd]	6.12±0.13[cd]	8.46±0.16[b]	30.98±1.14[cd]	33.32±1.30[cd]	6.34±0.30[ab]	3.38±0.21[b]	1.78±0.11[bc]
Raipur	2.66±0.12[c]	12.18±0.38[e]	2.08±0.11[e]	6.78±0.12[e]	5.46±0.13[ef]	7.64±0.16[cd]	29.16±1.01[d]	30.96±1.14[d]	6.72±0.32[a]	4.06±0.17[a]	2.10±0.08[a]
Bhubaneshwar	2.70±0.07[bc]	12.38±0.43[e]	2.12±0.11[de]	6.91±0.15[de]	5.16±0.24[f]	7.26±0.12[d]	29.27±1.22[d]	31.75±1.31[cd]	6.51±0.30[a]	4.13±0.18[a]	2.14±0.10[a]

*Mean±Standard deviation of five replications; means in a column followed by common letter (s) are not significantly different from each other (Tukey's HSD test, $p \leq 0.05$)

Table 3: Reproductive potential of female moths of *M. vitrata* populations collected from different locations across India

Locations	Pre-oviposition Period (days)	Oviposition period (days)	Post-oviposition period (days)	Fecundity (eggs/female)	Sex ratio (♂: ♀)
Ludhiana	2.76±0.08^{a}	3.98±0.08^{a}	2.90±0.12^{a}	62.84±5.77^{a}	1:1.40±0.09^{a}
Hisar	2.68±0.13^{ab}	3.80±0.07^{ab}	2.82±0.10^{ab}	56.88±3.68^{ab}	1:1.36±0.06^{ab}
New Delhi	2.64±0.11^{ab}	3.72±0.13^{b}	2.88±0.13^{a}	50.12±2.29^{bc}	1:1.30±0.08^{abc}
Pantnagar	2.24±0.05^{de}	3.30±0.07^{de}	2.40±0.15^{cd}	32.12±3.61^{f}	1:1.15±0.03^{d}
Kanpur	2.50±0.07^{bc}	3.52±0.08^{c}	2.60±0.12^{bc}	40.12±3.75^{de}	1:1.20±0.03^{cd}
Varanasi	2.38±0.08^{cd}	3.42±0.10^{cd}	2.66±0.11^{ab}	36.12±3.04^{ef}	1:1.25±0.06^{bcd}
Raipur	2.10±0.07^{e}	3.04±0.08^{f}	2.12±0.10^{e}	44.32±2.85^{cd}	1:1.18±0.04^{cd}
Bhubaneswar	2.16±0.11^{e}	3.20±0.10^{ef}	2.28±0.13^{de}	45.80±2.70^{cd}	1:1.20±0.05^{cd}

*Mean±Standard deviation of five replications; means in a column followed by common letter (s) are not significantly different from each other (Tukey's HSD test, $p \leq 0.05$)

Table 4: Egg morphometry of *M. vitrata* populations collected from different locations across India

Populations	Egg dimensions (mm)	
	L	W
Ludhiana	0.66±0.02^{ab}	0.46±0.02^{ab}
Hisar	0.65±0.02^{ab}	0.46±0.01^{ab}
New Delhi	0.65±0.01^{ab}	0.46±0.01^{ab}
Pantnagar	0.63±0.02^{b}	0.43±0.02^{c}
Kanpur	0.64±0.04^{b}	0.44±0.03^{bc}
Varanasi	0.64±0.01^{b}	0.45±0.00^{abc}
Raipur	0.68±0.01^{a}	0.48±0.01^{a}
Bhubaneswar	0.68±0.02^{a}	0.47±0.001^{a}

*Mean±standard deviation of five replications; means in a column followed by common letter (s) are not significantly different from each other (Tukey's HSD test, $p \leq 0.05$).

and sex ratio (1:1.40), while Pantnagar had the lowest fecundity (32.12±3.61 eggs per female) and a sex ratio of 1:1.15 (Table 3). Fecundity was reported to be 144.5±100.2 eggs per female (Savde *et al.,* 2018) and 32.16±2.70 eggs per female (Shejulpatil *et al.,* 2020). *M. vitrata* eggs are oval, milky white, and translucent when freshly laid (Fig. 1a), gradually transitioning to golden yellowish cream (Fig. 1b) and later golden yellow with maturation and the anterior end turns dark after 2-3 days (Fig. 1c). Eggs adhere to their substrates singly or in small groups and hatching takes place normally on the third day after oviposition. Morphometric analysis showed that there was significant variation in the egg sizes in populations within India (Table 4). The biggest eggs were found in the Bhubaneswar (0.68±0.02 mm, 0.48±0.01 mm) and the Raipur populations (0.68±0.01 mm, 0.47±0.001 mm). On the other hand, the smallest eggs were recorded from Pantnagar population (0.63±0.02 mm, 0.43±0.02 mm) (Table 4). These observations are in line with the previous works (Mahankuda and Tiwari, 2020; Shejulpatil *et al.,* 2020); Rachappa *et al.,* 2017).

The larval stage of *M. vitrata* has five instars with size and color variations (Fig. 2). Head capsule growth follows Dyar's law with a geometric increase. Body size varied significantly across locations, despite slight head capsule differences. Our study found notable variations in larval size and head capsule dimensions across different regions, especially in the first four instars (Table 5). First instar larvae are tube-shaped, translucent, off-white, with darker heads and prothoracic regions (Fig. 2a). Length varied between 1.24 (Pantnagar) and 1.31 mm (Bhubaneswar) and width between 0.15 (Pantnagar) and 0.19 mm (Bhubaneswar) (Table 1), and the head capsule widths were recorded to be 0.18 (Pantnagar) to 0.21 mm (Ludhiana, New Delhi, Raipur and Bhubaneswar) (Table 5).

The larvae turned dirty white with brown sclerites, and their heads and prothorax darkened further (Fig. 2b). The dimensions for this stage ranged from 2.46 (Pantnagar) to 2.82 mm (Bhubaneswar) in length, 0.35 (Pantnagar) to 0.41 mm (Raipur and Bhubaneswar) in width, and 0.32 (Pantnagar) to 0.35 mm (Raipur and Bhubaneswar) for the head capsule width (Table 5). Third instar larvae were distinguishable by their prominent black spots and progressive increase in the body length (Fig. 2c). Their dimensions ranged from 5.25 (Pantnagar) to 5.40 mm (Bhubaneswar) in length, 0.80 (Pantnagar) to 0.88 mm (Bhubaneswar) in width, and head capsule widths of 0.53 (Pantnagar) to 0.58 mm (Bhubaneswar), respectively (Table 5).

Fourth instar larvae were creamy white or sometimes light greenish, with deep dark spots and dark brown heads (Fig. 2d). Dimensions ranged from 10.67 (Pantnagar) to 11.48 mm (Bhubaneswar) in length, 1.65 (Pantnagar) to 1.76 mm (Bhubaneswar) in width, and 0.80 (Pantnagar) to 0.86 mm (Bhubaneswar) for the head capsule width, respectively (Table 5). The fifth and final instar larvae attained maximum size, with body length ranging from 16.13 (Pantnagar) to 16.70 mm (Bhubaneswar), widths of 2.34 (Pantnagar) to 2.46 mm (Bhubaneswar), and head capsule widths of 1.28 (Pantnagar) to 1.38 mm (Bhubaneswar) (Table 5; Fig. 2e). Overall, the Bhubaneswar population recorded the maximum larval body

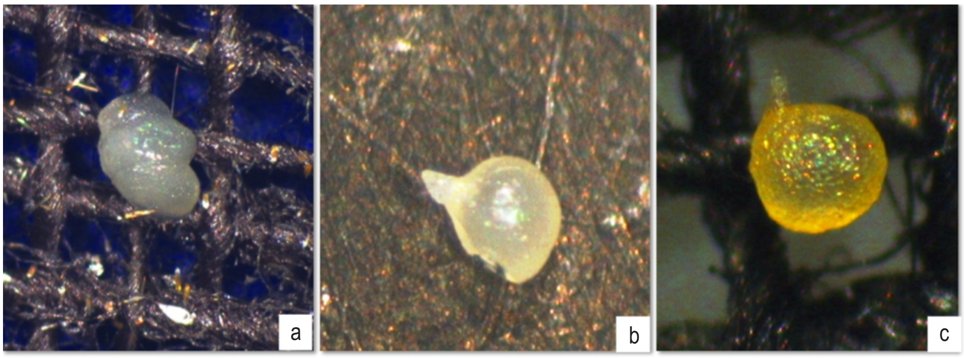

Fig. 1: Colour change in *M. vitrata* eggs, (a): Freshly laid (milky white), (b): After 14-36 hours of deposition (off white coloured), (c): Just prior to hatching (golden yellow); Magnification 25X

Fig. 2: Different larval instars of *M. vitrata*, (a): First instar, (b): Second instar, (c): Third instar, (d): Fourth instar and (e): Fifth instar.

length and width across all instars, which was statistically at par with the populations from Raipur, Ludhiana, and Hisar. In contrast, the Pantnagar population had the smallest larval body size. The larval chaetotaxy of *M. vitrata* were consistent with Crambidae (Fig. 3). Key features includedone L seta on A9 and two pinacula without setae on the meso- and meta-thoracic (T2-3) segments located posteriorly with respect to the D pinacula. In addition, the pre-spiracular pinaculum of T1 was crescent-shaped and positioned lower than the spiracle, whereas the SD1 pinacula of A2 and A7 were not reduced (Fig. 3). No pinacula without setae was observed in the abdomen. In fully grown larvae, ten main setae were observed on the prothoracic shield: two dorsal setae (D1,D2) with two others (XD1, XD2), subdorsal setae (SD1, SD2), lateral (L1, L2), and subventral (SV1, SV2) (Fig. 3). These setae exhibited a common pattern across other geographical locations regardless of regional differences, which explains why similarity was found in larval identification.

This study is consistent with the previous studies (Hanabar and Hegde, 2018; Shejulpatil *et al.,* 2020; Sravani and Mahalakshmi, 2016), and it may be inferred that both environmental and host factors affect larval growth. Geographic variations in *M. vitrata* populations across India is similar to that found globally that shows the host plant quality and climatic conditions affect the developmental rates (Jackai, 1995; Okeyo-Owuor and Oloo, 1991). Host-specific nutrition may also influence the developmental schedule (Keerthi *et al.,* 2018). Our observations showed a consistent pattern across geographic locations, supporting the standardization of *M. vitrata* larval identification diagnostic key described by Gillian and Passoa (2014). The obtect pupae were enclosed in a gauze-like cocoon, and exhibit 360° segment rotation under stimulation (Fig. 4). Significant differences in the size and weight of pupawere observed across populations (Table 5). Bhubaneswar had the largest pupae in terms of length (11.75 mm), width (2.92 mm), and

Table 5: Larval and pupal morphometry of *M. vitrata* populations collected from different locations across India

Populations	First instar larva			Second instar larva			Third instar larva			Fourth instar larva			Fifth instar larva			Pupa		
	L	W	HCW	L	W	HCW	L	W	HCW	L	W	HCW	L	W	HCW	L	W	Wgt
Ludhiana	1.28± 0.01^{abc}	0.18± 0.01^{ab}	0.21± 0.00^{a}	2.63± 0.04^{cd}	0.39± 0.02^{abc}	0.34± 0.00^{ab}	5.37± 0.02^{ab}	0.84± 0.01^{b}	0.57± 0.01^{ab}	11.33± 0.07^{ab}	1.75± 0.01^{ab}	0.84± 0.01^{abc}	16.44± 0.03^{bc}	2.43± 0.02^{abc}	1.34± 0.01^{bc}	11.72± 0.01^{ab}	2.90± 0.01^{ab}	47.46± 1.68^{ab}
Hisar	1.26± 0.02^{cd}	0.17± 0.01^{abc}	0.20± 0.00^{a}	2.60± 0.02^{d}	0.38± 0.02^{abcd}	0.34± 0.01^{ab}	5.34± 0.02^{abc}	0.83± 0.01^{bc}	0.56± 0.00^{ab}	11.25± 0.06^{bc}	1.73± 0.01^{ab}	0.83± 0.01^{ac}	16.40± 0.07^{bc}	2.42± 0.03^{abc}	1.33± 0.01^{bc}	11.70± 0.02^{ab}	2.88± 0.02^{abc}	46.06± 2.03^{bc}
New Delhi	1.27± 0.01^{bc}	0.18± 0.01^{ab}	0.21± 0.00^{a}	2.71± 0.04^{bc}	0.39± 0.02^{abc}	0.34± 0.01^{ab}	5.33± 0.02^{abc}	0.85± 0.01^{ab}	0.56± 0.01^{ab}	11.12± 0.04^{c}	1.74± 0.02^{ab}	0.83± 0.00^{abc}	16.53± 0.07^{ab}	2.41± 0.01^{bcd}	1.31± 0.02^{cd}	11.68± 0.03^{abc}	2.85± 0.03^{bcd}	45.12± 1.60^{bcd}
Pantnagar	1.24± 0.00^{d}	0.15± 0.01^{c}	0.18± 0.01^{b}	2.46± 0.03^{f}	0.35± 0.00^{d}	0.32± 0.01^{b}	5.25± 0.06^{d}	0.80± 0.03^{c}	0.53± 0.00^{c}	10.67± 0.06^{e}	1.65± 0.03^{d}	0.80± 0.02^{d}	16.13± 0.05^{d}	2.34± 0.03^{e}	1.28± 0.01^{d}	11.58± 0.04^{d}	2.76± 0.03^{e}	39.80± 1.59^{d}
Kanpur	1.26± 0.01^{cd}	0.16± 0.00^{bc}	0.19± 0.01^{ab}	2.50± 0.05^{ef}	0.36± 0.01^{cd}	0.33± 0.00^{ab}	5.29± 0.03^{cd}	0.82± 0.00^{bc}	0.54± 0.01^{bc}	10.80± 0.08^{de}	1.67± 0.01^{cd}	0.81± 0.01^{cd}	16.29± 0.09^{c}	2.37± 0.01^{de}	1.29± 0.02^{d}	11.62± 0.03^{cd}	2.80± 0.03^{de}	41.56± 1.77^{d}
Varanasi	1.26± 0.01^{cd}	0.16± 0.01^{abc}	0.20± 0.00^{a}	2.56± 0.04^{de}	0.37± 0.03^{bcd}	0.34± 0.01^{ab}	5.32± 0.03^{bc}	0.83± 0.01^{bc}	0.54± 0.00^{bc}	10.95± 0.09^{d}	1.72± 0.03^{bc}	0.82± 0.02^{bcd}	16.36± 0.08^{bc}	2.40± 0.02^{cd}	1.30± 0.00^{cd}	11.66± 0.05^{bc}	2.82± 0.02^{cde}	43.54± 1.95^{cd}
Raipur	1.30± 0.02^{ab}	0.18± 0.01^{ab}	0.21± 0.00^{a}	2.73± 0.06^{ab}	0.41± 0.01^{ab}	0.35± 0.01^{a}	5.38± 0.04^{ab}	0.86± 0.02^{ab}	0.57± 0.02^{a}	11.42± 0.04^{a}	1.75± 0.01^{ab}	0.85± 0.01^{ab}	16.65± 0.05^{a}	2.45± 0.01^{ab}	1.36± 0.01^{ab}	11.73± 0.03^{ab}	2.90± 0.02^{ab}	49.38± 1.09^{ab}
Bhubaneswar	1.31± 0.01a	0.19± 0.01^{a}	0.21± 0.01^{a}	2.82± 0.04^{a}	0.41± 0.00^{a}	0.35± 0.02^{a}	5.40± 0.02^{a}	0.88± 0.02^{a}	0.58± 0.01^{a}	11.48± 0.08^{a}	1.76± 0.02^{ab}	0.86± 0.00^{a}	16.70± 0.06^{a}	2.46± 0.01^{a}	1.38± 0.02^{a}	11.75± 0.02^{a}	2.92± 0.03^{a}	51.18± 1.49^{a}

*Mean±Standard deviation of five replications; means in a column followed by common letter (s) are not significantly different from each other (Tukey's HSD test, $p \leq 0.05$). L: Length (mm), W: Width (mm), HCW: Head capsule width (mm), Wgt: Weight (mg).

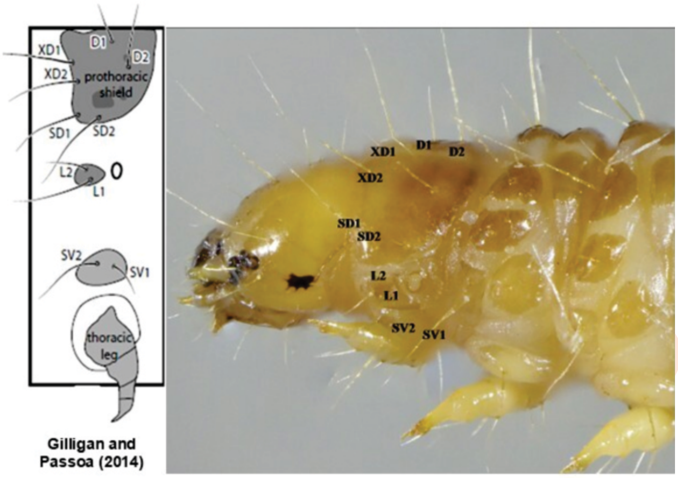

Fig. 3: Setal arrangement on prothoracic segment of *M. vitrata* larva.

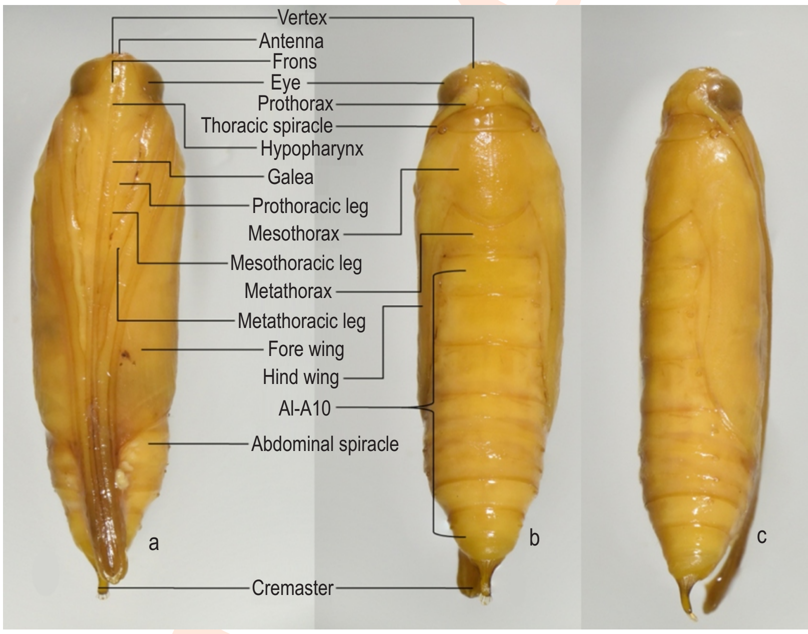

Fig. 4: Pupa of *M. vitrata*, (a): Ventral view, (b): Dorsal view and (c): Lateral view.

weight (51.18 mg), followed by Raipur, Ludhiana, Hisar, and New Delhi populations. Pantnagar recorded the smallest pupae, with a length of 11.58 mm, width of 2.76 mm, and weight of 39.80 mg (Table 5). Males had a tapering dark grey abdomen with black hairs at the tip (Fig. 5a,b), whereas females had a tubular abdomen ending in a bifid hairy ovipositor (Fig. 5c,d). Body coloration was yellowish-brown to fuscous brown (Fig. 5). Significant differences in body length were observed across the populations. Bhubaneswar recorded the longest body lengths for both males (12.06±0.16 mm) and females (13.04±0.17 mm), while Pantnagar had the shortest lengths for males (10.92±0.21 mm) and females (11.68±0.14 mm) among the studied populations (Table 6).

Fore wings were fuscous brown with three white spots surrounded by black margins on the upper half of the wing, and the coastal area was tinged fulvous (Fig. 6a). The hind wings were partially hyaline and milky white, with the terminal quarter having

Table 6: Adult morphometry of *M. vitrata* populations collected from different locations across India

Populations	Male moth (mm)						Female moth (mm)			
	Body Length	Hind Wing Length	Aedeagus length	Valva length	Saccus length	Genitalis length	Body Length	Hind Wing Length	Bursa copulatrix	Ovipositor lobe width
Ludhiana	11.60±0.17^{bc}	7.82±0.08^{bc}	1.28±0.01^{ab}	2.08±0.06^{ab}	0.38±0.06^{a}	1.47±0.07^{bc}	12.65±0.28^{ab}	8.68±0.10^{bc}	2.17±0.03^{ab}	0.41±0.02^{ab}
Hisar	11.51±0.23^{bc}	7.72±0.08^{bc}	1.27±0.03^{ab}	2.05±0.02^{ab}	0.38±0.04^{a}	1.46±0.05^{bc}	12.48±0.21^{bc}	8.52±0.08^{cd}	2.16±0.03^{ab}	0.40±0.03^{ab}
New Delhi	11.35±0.22^{cd}	7.65±0.09^{cd}	1.27±0.04^{ab}	2.06±0.06^{ab}	0.37±0.02^{a}	1.46±0.02^{c}	12.17±0.25^{cd}	8.36±0.10^{de}	2.17±0.03^{ab}	0.39±0.03^{ab}
Pantnagar	10.92±0.21^{e}	7.35±0.07^{f}	1.23±0.02^{b}	1.88±0.03^{c}	0.33±0.04^{a}	1.36±0.03^{d}	11.68±0.14^{e}	7.96±0.09^{g}	2.02±0.07^{c}	0.36±0.02^{b}
Kanpur	11.10±0.15^{de}	7.40±0.09^{ef}	1.25±0.02^{ab}	1.94±0.03^{c}	0.34±0.03^{a}	1.40±0.02^{cd}	11.76±0.23^{de}	8.07±0.09^{fg}	2.08±0.07^{bc}	0.37±0.02^{ab}
Varanasi	11.25±0.16^{cde}	7.51±0.12^{de}	1.26±0.00^{ab}	1.98±0.06^{bc}	0.35±0.03^{a}	1.42±0.04^{cd}	11.90±0.16^{de}	8.19±0.07^{ef}	2.13±0.04^{abc}	0.38±0.01^{ab}
Raipur	11.83±0.13^{ab}	7.89±0.12^{ab}	1.30±0.04^{a}	2.11±0.03^{a}	0.39±0.03^{a}	1.55±0.03^{ab}	12.84±0.21^{ab}	8.81±0.06^{b}	2.19±0.05^{a}	0.42±0.04^{a}
Bhubaneswar	12.06±0.16^{a}	8.07±0.10^{a}	1.30±0.02^{a}	2.12±0.04^{a}	0.40±0.03^{a}	1.58±0.04^{a}	13.04±0.17^{a}	9.03±0.08^{a}	2.20±0.04^{a}	0.43±0.02^{a}

*Mean±standard deviation of five replications; means in a column followed by common letter (s) are not significantly different from each other (Tukey's HSD test, $p \leq 0.05$.

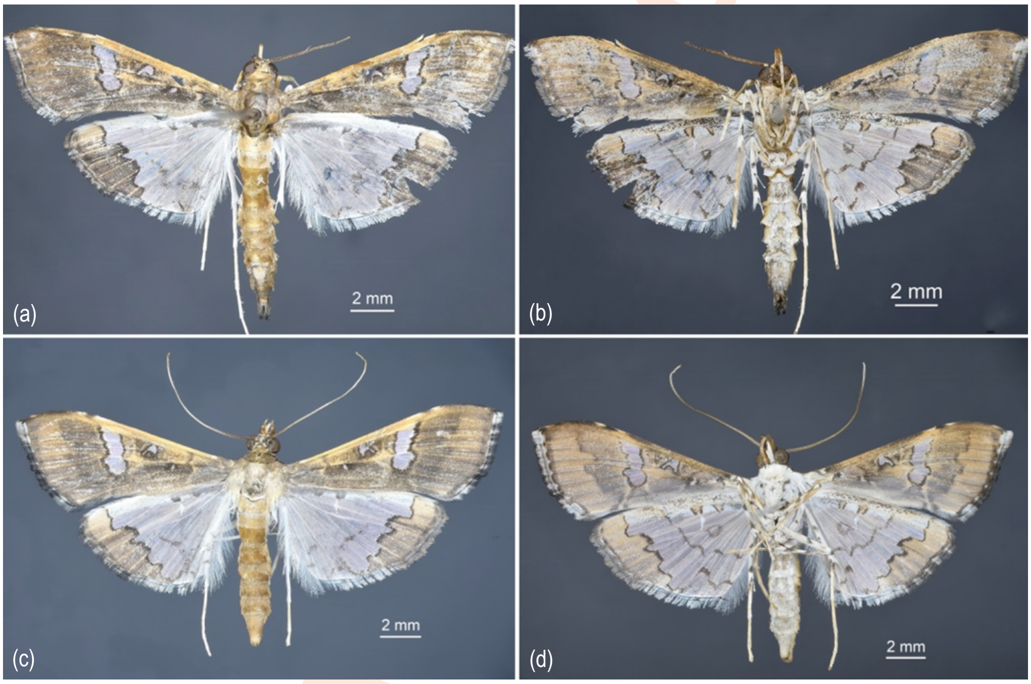

Fig. 5: *M. vitrata* adults, (a): Male - dorsal view, (b): Male - ventral view, (c): Female - dorsal view, (d): Female - ventral view.

a yellowish coastal margin and a dark brown apical patch with a spot at the upper angle of the cell (Fig. 6b). The fore and hind wings had 14 longitudinal and eight longitudinal veins (Fig. 7). Bhubaneswar recorded the longest hind wings for both males (8.07±0.10 mm) and females (9.03±0.08 mm), followed by the Raipur (males: 7.89±0.12 mm; and females: 8.81±0.06 mm) (Table 6). In contrast, Pantnagar recorded the shortest wingspan of the hind wings (males: 7.35±0.07 mm; and females: 7.96±0.09 mm) (Table 6). The results are consistent with the findings of Shejulpatil *et al.* (2020) and Shinde *et al.* (2017), who also reported larger body and wing dimensions among females (11.05-11.88 mm body length and 24.70-24.98 mm wing expanse) than males (9.8-11.10 mm body length and 20.30-24.38 mm wing expanse) when reared on different hosts, suggesting that the morphometry of adult moths is influenced by geographical and local environmental conditions. In the current study, irrespective of the location, both fore wings and hind wings were consistently longer in females than males across locations.

Female (bursa copulatrix and ovipositor lobe width) and mature male (aedeagus, valva, saccus, and corpus genitalis) *M. vitrata* genitalia varied throughout India (Fig. 8). The male

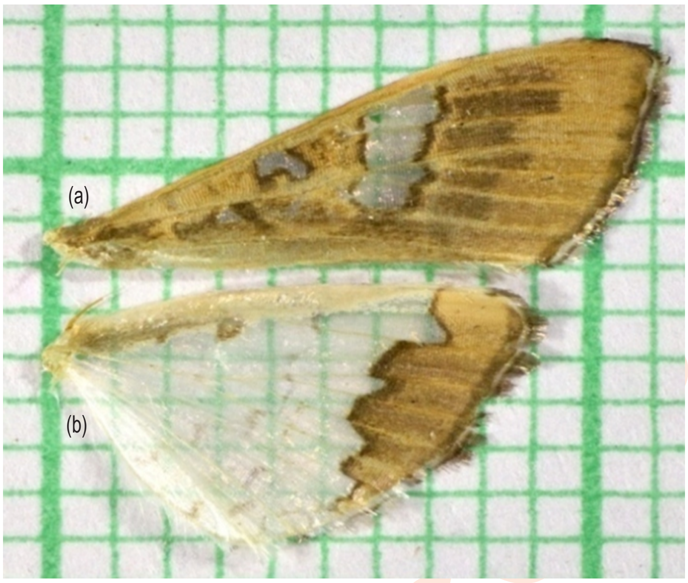

Fig. 6: Wings of *M. vitrata,* (a): Fore wing and (b): Hind wing.

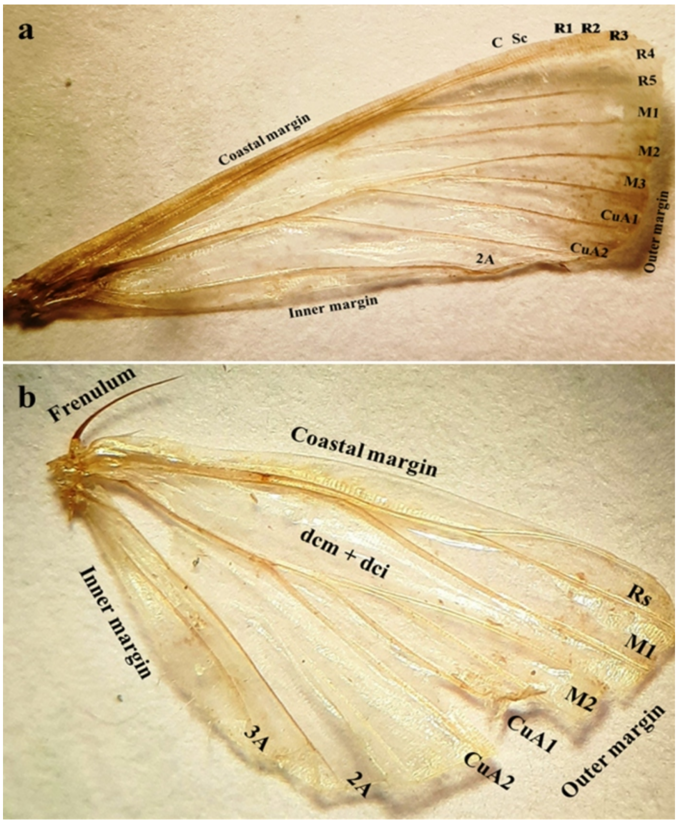

Fig. 7: Wing venation of *M. vitrata,* (a): Fore wing and (b): Hind wing; Costa (c), Subcosta (Sc), Radius (R), Media (M), Cubitus (Cu), Anal (A),Upper discocellular (dcs), Median discocellular (dcm) and Lower discocellular (dci).

(a) (b)

Fig. 8: Genitalia of *M. vitrata,* (a) Male; (b) Female (Magnification 35X).

population in Bhubaneswar had the longest aedeagus, valva, and corpus genitalis (Table 6), followed by Raipur. In Pantnagar, the shortest measures were observed, including aedeagus length of 1.23±0.02 mm, valva length of 1.88 mm, and corpus genitalis length of 1.36±0.03 mm (Table 6 shows that saccus length was similar among populations (0.33-0.40 mm)). Female bursa copulatrix and ovipositor lobes varied widely (Table 6). Table 6 shows that the bursa copulatrix and ovipositor lobe were longest in Bhubaneswar (2.20±0.04 mm and 0.43±0.02 mm, and lowest in Pantnagar (2.02±0.07 mm and 0.36±0.02 mm, Kirti and Gill (2005) separated *M. amboinalis* from *M. vitrata* based on their external genitalia. Kumar *et al.* (2013) also illustrated the taxonomic diagnostic tools for identifying *M. vitrata* based on the external genital features. However, no previous studyhas documented the measurement of genital morphometric in *M. vitrata*.

Regional differences in *M. vitrata* developmental periods and morphometry are affected by abiotic factors and host plant-types at all life stages. Extended developmental stages represent favourable environmental conditions that contribute to improvement in reproduction and growth rates. These findings suggest that consideration of regional morphometric variations is important in formulation of IPM programmes. Timely and targeted interventions are crucial to prevent exponential growth of substantial and faster growing population of *M. vitrata,* necessitating its location-specific management tactics for effective control. This study shows that the morphometric and developmental traits of *M. vitrata* vary regionally in India indicating heritable intraspecific variation in development, reproduction and morphology. Temperature and humidity have a significant effect on insect growth and development (Sibly and Atkinson, 1994; Marri *et al.,* 2023). Greater larval stage duration, shorter pupation period and changing female/male ratios were observed in populations. Populations showed increased larval developmental time, decreased pupation time and modified female/male ratio. These results should be field-verified since the experiments were performed in controlled laboratory conditions. An understanding of the biology of *M. vitrata* is important for successful IPM implementation, since this has provided the basis for the timing of interventions to minimize resistance risks. This study offers valuable information on *M. vitrata* development and diversity for sustainable IPM of pigeonpea production in India by early biology-based interventions.

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photography and manuscript revision; **S. Premkumari:** Data compilation and analysis; **S. Chakravarty:** Manuscript revision; **K.G. Padwal:** Manuscript revision and **C.P. Srivastava:** Corresponding author, supervision of research and guidance in drafting the manuscript.

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