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Biology of zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fabricius) (Coccinellidae: Coleoptera) on Cowpea aphid, *Aphis craccivora* (Koch) (Aphididae: Hemiptera)

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Abstract

An investigation on the biology of zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fabricius) on cowpea aphid, *Aphis craccivora* (Koch) was conducted under laboratory conditions. The results revealed that the mean incubation period was 1.64 ± 0.72 days. The mean hatching percentage of eggs was 89.25 ± 3.97 per cent. The mean duration of first, second, third and fourth instar larva was 1.26 ± 0.44 , 1.68 ± 0.59 , 2.28 ± 0.54 and 2.44 ± 0.61 days, respectively. The total larval period was 7.66 ± 1.61 days. The mean prepupal and pupal period was 1.04 ± 0.20 and 2.18 ± 0.48 days, respectively. The mean percentage of adult emergence was 89.48 ± 4.44 per cent with a sex ratio of 1:1.40. The mean pre-oviposition, oviposition and post-oviposition was 3.48 ± 0.77 , 16.40 ± 1.29 and 3.00 ± 0.87 days, respectively. The fecundity of a gravid female was 363.32 ± 76.32 eggs. The adult longevity in male and female adults was 17.12 ± 1.44 days and 22.54 ± 2.38 days, respectively. The total life span of males was 29.64 ± 2.59 while in the females it was 35.06 ± 3.17 days.

Keywords: Biology, morphometrics, *Cheilomenes sexmaculata*, *Aphis craccivora*, coccinellid, cowpea aphid

1. Introduction

Biological control is the beneficial action of parasites, pathogen and predators in managing pests and their damage. Biological control is often viewed as an economic and sustainable way to control pest organisms. When effective, it can give practically permanent, extensive control with a very favourable cost-benefit ratio (Bale *et al.*, 2008) [2]. Coccinellids are no exceptions to the trend and they constitute the major group of predatory insects feeding on a wide range of soft bodied insects including aphids, plant hoppers, thrips, jassids, scale insects, mealy bugs and whitefly infesting large number of cultivated crops (Omkar and Pervez, 2004) [7]. The utilization of coccinellids in biological control has been roller coaster ride since its first successful attempt to control cotton cushiony scale *Icerya purchasi* (Maskell) by vedalia beetle *Rodolia cardinalis* (Mulsant). The Zigzag ladybird, *Cheilomenes sexmaculata* (Fabricius) is the well-known species of coccinellid found on all over the vegetation in India. It has the ability to control aphid populations in many different agricultural ecosystems. Studies on the biology of the predator provide information on various stages and their behavior which can assist in identifying the predatory stage, establishing prey predator relationship and potential of its life stages for better pest management. Hence the present investigation was carried out to study the life history parameters of *C. sexmaculata* on *Aphis craccivora* (Koch).

2. Material and Methods

The biology of *C. sexmaculata* was examined on *A. craccivora* under laboratory conditions in Post Graduate Research Laboratory of Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari (Gujarat) during January 2022 to March 2022 at 27.18 ± 1.45 °C temperature and 49.80 ± 7.64 per cent relative humidity. The nucleus culture of aphid, *A. craccivora* and the ladybird beetle, *C. sexmaculata* were collected from fields and maintained on Indian Bean (*Dolichos lablab* L.) under laboratory.

A total of 50 eggs of *C. sexmaculata* were collected from the stock culture after 24 h of egg laying with a soft and moist camel brush. The collected eggs were reared individually in plastic vials (6 x 4 cm) containing the aphids as food. They were allowed to feed throughout their life. The length and breadth of thirty individuals of different stages of the predator were calculated with the help of stereo-trinocular microscope fitted with Catcam-130 camera having software Scopephoto. The incubation period and the hatching percentage of the eggs were

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noted. The number of larval instars and duration were determined on the basis of exuviae casted off and recording days between two successive moulting. The pre-pupal stage was determined when the fourth instar larva stopped feeding and became sluggish to the time when it turned to pupa. Later, the pupal period was determined. The newly emerged 50 adults of *C. sexmaculata* were observed to record the longevity of male and female, separately. A total of 25 pairs of *C. sexmaculata* were placed individually in the plastic vials to record the data on fecundity, pre-oviposition, oviposition, post-oviposition period of females and sex-ratio (male: female).

3. Results and Discussion

The results obtained on morphometrics and various biological parameters of the zigzag ladybird beetle, *C. sexmaculata* have been presented in Table 1 and Table 2.

3.1 Egg

The female adult of *C. sexmaculata* laid eggs singly or in clusters of 3-20 eggs near the aphid colonies, on twigs and on the walls of the container. The freshly laid eggs of *C. sexmaculata* were bright yellowish in colour, cigar shaped with smooth chorion without any reticulations (Fig. 1). The eggs turned to blackish in colour with the advancement of age and became completely black before hatching. The length of the eggs varied from 0.77-1.12 mm with a mean 1.02 ± 0.08 mm, whereas the width varied from 0.34-0.52 mm with a mean of 0.43 ± 0.04 mm (Table 1). The present findings are in agreement with Tank and Korat (2007)^[17] who reported that the mean length and breadth of eggs of *C. sexmaculata* was 1.01 ± 0.11 mm and 0.39 ± 0.03 mm, respectively when reared on *Aphis gossypii* (Glover). Similarly, when reared on *A. craccivora*, the average length and breadth of the egg as reported by Rai *et al.* (2003)^[11] was 1.05 ± 0.01 mm and 0.49 ± 0.01 mm, while, Shinde (2012)^[14] mentioned that the length and breadth varied from 0.79 to 1.10 mm (mean, 1.01 ± 0.10 mm), and 0.38 to 0.47 mm (mean, 0.43 ± 0.03 mm), respectively.

3.1.2 Incubation period

The incubation period of *C. sexmaculata* ranged from 1 to 3 days with a mean of 1.64 ± 0.72 days (Table 2). Similar results were obtained by Rai *et al.* (2003)^[11] and Tank and Korat (2007)^[17] with an average incubation period of 1.68 ± 0.16 and 1.40 ± 0.66 days, respectively.

3.1.3 Hatching percentage

The hatching percentage of eggs of *C. sexmaculata* presented in Table 2 ranged from 80.00 to 93.94 per cent with a mean of 89.25 ± 3.97 per cent. The data were more or less in accordance with the findings of Tank and Korat (2007)^[17] who reported 85.96 per cent egg hatchability when reared on *A. gossypii*. More or less similar observations were made by Patel (2015)^[9] who reported that the hatchability of *C. sexmaculata* eggs varied from 80-92 per cent when reared on different aphid species, while Priyadarshani *et al.* (2016)^[10] found 84 to 88 per cent with an average of 85.86 per cent hatchability when reared on *A. craccivora*.

3.2 Larva

The larvae of *C. sexmaculata* moulted three times, resulting in four larval instars when reared on *A. craccivora*. This is corroborated with the reports of earlier workers (Rai *et al.*,

2003^[11]; Tank and Korat, 2007^[17]; Singh *et al.*, 2008; Shinde, 2012^[14]; Routray *et al.*, 2016^[12]; Mishra *et al.*, 2021)^[4].

3.2.1 First instar

The newly hatched first instar larva was dark greyish in colour with shiny black head capsule and greyish legs that eventually turned black (Fig 1). The larval body was covered with spines. The length of the first instar larvae ranged from 1.23 to 1.89 mm with a mean of 1.58 ± 0.15 mm and the breadth ranged between 0.34-0.52 mm with a mean of 0.43 ± 0.04 mm (Table 1). The present results are more or less similar to the findings of Tank and Korat (2007)^[17] with the mean length and breadth of 1.41 ± 0.16 mm and 0.42 ± 0.02 mm, respectively. Shanmugapriya *et al.* (2017)^[13] noticed the mean length and breadth as 1.41 ± 0.22 mm and 0.49 ± 0.05 mm, respectively when reared on *Lipaphis erysimi* (Kaltenbach). The duration of first instar larva varied from 1 to 2 days with a mean of 1.26 ± 0.44 days (Table 2). The present findings are consistent with the reports of Omkar and Bind (2004)^[6] on *A. craccivora*, who found that the duration of first instar larvae was 1.39 ± 0.16 days. More or less similar findings were noticed by Rai *et al.* (2003)^[11] on *A. craccivora* with the first instar larval duration as 1.6 ± 0.15 days, while Tank and Korat (2007)^[17] reported it as 1.80 ± 0.50 days when fed on *A. gossypii*. The slight variation in the results could be due to the variation in prey species or host plants used and the experimental conditions under which the study was conducted.

3.2.2 Second instar

The freshly mounted second instar larva was long, slender, shiny black with yellow head capsule and legs which turned to black gradually. White patches were found on the meso and metathorax, as well as the dorsolateral side of the first and fourth abdominal segments (Fig. 1). The larva was flat ventrally, with a slightly convex dorsal body. The abdominal segments were wider near the thoracic area and tapering towards the end. Similar descriptions were given by Tank and Korat (2007)^[17] and Routray *et al.* (2016)^[12]. It was noticed that the length of the second instar larvae varied from 3.25 to 4.59 mm with a mean length of 4.01 ± 0.32 mm, while the breadth ranged between 0.64-0.96 mm with a mean of 0.79 ± 0.1 mm (Table 1). The present findings are in close association with the results of Shinde (2012)^[14] on *A. craccivora* where the length and breadth of second instar larva varied between 3.84 to 4.56 mm (mean, 4.17 ± 0.22 mm) and 0.67 to 0.87 mm (mean, 0.76 ± 0.06 mm), respectively. The duration of second instar lasted for 1 to 3 days, with a mean of 1.68 ± 0.59 days (Table 2). The results are in close conformity with the findings of Shinde (2012)^[14] and Pandi *et al.* (2012)^[8] who reported that the duration of second instar was 1.70 ± 0.24 and 1.6 ± 0.10 days, respectively when reared on *A. craccivora*.

3.2.3 Third instar

The third instar larva was dark grey to black in colour with yellow head capsule and conspicuous spiny structures on the body. The white patches were intensified on the meso and metathorax and the dorso-lateral sides of first and fourth abdominal segments with additional development of dark grey patches on mid-dorsal line of abdominal segments (Fig. 1). The length of third instar larva of *C. sexmaculata* ranged between 4.75-6.08 mm with a mean of 5.41 ± 0.35 mm while

the breadth was 1.05-1.26 mm with a mean of 1.14 ± 0.05 mm (Table 1). These results are in agreement with the findings of Chakraborty (2012) ^[3] who noticed that the length of third instar larva ranged between 4.76-5.47 mm (mean, 5.46 ± 0.23 mm) and breadth was 1.72-1.82 mm (mean, 1.77 ± 0.03 mm) and Shinde (2012) ^[14] reported that the length and breadth of the third instar varied between 4.76-5.47 mm (mean, 5.13 ± 0.23 mm) and 0.92-1.24 mm (mean, 1.06 ± 0.10 mm). The duration of third instar lasted for 2-4 days with a mean of 2.28 ± 0.54 days (Table 2). The present findings are in accordance with the results of Routray *et al.* (2016) ^[12] who found that the duration of third instar was 2.25 ± 0.86 days when fed with *A. craccivora* on greengram. Similar observations were recorded by Shinde (2012) ^[14] who reported the duration of third instar ranged between 2.10-2.61 days when fed with different aphids. The observations on the duration of third instar by Mishra *et al.* (2021) ^[4] varied from 2.5 to 2.9 days during different months of the year. This implies that the variation in the results are due to temporal, demographic changes and also the prey species on which the test insect was reared.

3.2.4 Fourth instar

The freshly moulted fourth instar larva was deep black in colour which changed to dull black before pupation (Fig. 1). The white patches expanded wider as the larva grew stouter with age. The length of the fourth instar varied from 6.34 to 7.36 mm with a mean of 6.91 ± 0.33 mm, while the breadth ranged between 1.29-1.67 mm with a mean of 1.48 ± 0.09 mm (Table 1). The present results are in tally with the findings of the earlier workers. Shinde (2012) ^[14] found that the length and breadth of the fourth instar larva varied between 6.67 to 7.34 mm (mean, 7.02 ± 0.19 mm) and 1.21 to 1.58 mm (mean, 1.34 ± 0.10 mm), respectively. Routray *et al.* (2016) ^[12] mentioned that the average length and breadth of the fourth instar larva was 7.03 ± 0.19 mm and 1.44 ± 0.25 mm. It was observed that the duration of fourth instar larva varied from 2 to 4 days with a mean of 2.44 ± 0.61 days (Table 2). Similar results were obtained by Pandi *et al.* (2012) ^[8] who found that the duration of fourth instar larva was 2.50 ± 0.15 days when fed with *A. craccivora*. Abbas *et al.* (2020) ^[1] reported the same as 2.47 ± 0.12 days when reared on *Diuraphis noxia* (Kurdjumov).

3.2.5 Total larval period

The total larval period ranged between 6 to 12 days with a mean of 7.66 ± 1.61 days (Table 2). The present findings are more or less similar with the reports of Tank and Korat (2007) ^[17] who found that the total larval period was 7.36 ± 1.22 days when reared on *A. gossypii*. Similarly, the grand mean of total larval period was 7.39 ± 0.18 days when fed with *A. craccivora* reared different host plants (Routray *et al.*, 2016) ^[12].

3.3 Prepupa

The fully developed fourth instar larva stopped feeding and remained sluggish, with a bloated body searching for a suitable place for pupation. The larva adhered its posterior abdominal segment to the walls of plastic vials or the leaf surface and assumed a curved shape to become pupa. The prepupa resembled the fourth instar with white patches visible on the larval body (Fig. 1). The prepupal period lasted for 1-2 days with a mean of 1.04 ± 0.20 days (Table 2). The present results are in conformity with the findings of Shinde (2012) ^[14] who reported that the prepupal was 1.04 ± 0.124 days when

reared on *A. craccivora*.

3.4 Pupa

The freshly formed pupa was bright yellow in colour, eventually turning to pale orange yellow. The fully grown pupa was yellowish orange in colour with a series of symmetrical triangular spots in two vertical lines on the dorsal side (Fig 1). The length and breadth of the pupa ranged between 2.92-3.79 mm and 1.86-2.70 mm with a mean of 3.29 ± 0.24 mm and 2.21 ± 0.18 mm, respectively (Table 1). The results are in close agreement with the findings of Shinde (2012) ^[14] who reported that the length of the pupa ranged between 3.09-3.48 with a mean of 3.28 ± 0.14 mm and the breadth varied between 1.97-2.42 mm with a mean of 2.21 ± 0.13 mm. It was observed that the pupal duration varied between 2-4 days with a mean of 2.18 ± 0.48 days (Table 2). More or less similar observations were recorded by Pandi *et al.* (2012) ^[8] who reported that the pupal duration was 2.5 ± 0.15 days while Rai *et al.* (2003) ^[11] reported it as 2.68 ± 0.14 days on *A. craccivora*.

3.5 Adult

The newly emerged adult was soft bodied, oval, convex dorsally and flat ventrally, translucent yellow without any markings on the elytra, which later developed into yellowish to orange red beetle with two Zig Zag black lines and a posterior black spot after sclerotisation and melanisation (Fig 1). The pronotum was pale yellow with T shaped black band connecting to the posterior black band. The females can be differentiated from the males by their larger size and their abdomen. The length and breadth of the male adults varied between 3.76-4.22 mm and 3.07-3.35 mm with a mean of 3.96 ± 0.12 mm and 3.25 ± 0.06 mm, while in females it was 4.76-5.48 mm and 3.55-4.26 mm with a mean of 5.13 ± 0.19 and 3.98 ± 0.20 mm, respectively (Table 1). A more or less similar results were given by Shinde (2012) ^[14] who mentioned that the length of female and male ranged between 3.61-4.57 mm (mean, 4.05 ± 0.27) and 4.66-5.36 (mean, 5.06 ± 0.22), while the breadth was 2.84-3.51 (mean, 3.22 ± 0.23) and 3.84-4.17 (mean, 4.02 ± 0.20), respectively. The current findings are further supported by the results of Routray *et al.* (2016) ^[12].

3.5.1 Adult emergence

The per cent adult emergence varied from 81.82 to 100 per cent with a mean of 89.48 ± 4.44 per cent (Table 2). Omkar and Bind (2004) ^[6] reported 71.80 per cent of adult emergence when reared on *A. craccivora*. The difference in the result could be due to temporal and geographic variations and the experimental conditions under which the study was conducted.

3.5.2 Sex ratio

The data pertaining to sex ratio revealed that out of 491 adults observed 206 were males and 287 were females and the sex ratio ranged between 1:1.22-1:1.71 with a mean of 1:1.40 (Table 2). This implies the predominance of females over the male adults. These results are in accordance with the earlier findings of Shinde (2012) ^[14] who reported that the average sex ratio was 1:1.39. A more or less similar results were obtained by Tank and Korat (2007) ^[17], Chakraborty (2012) ^[3] and Shanmugapriya *et al.* (2017) ^[13] where the sex ratio ranged between 1:1.30-1:1.45.

3.5.3 Pre-oviposition period

The pre-oviposition period of *C. sexmaculata* varied between 3-6 days with a mean of 3.48±0.77 days (Table 2). The present results were in agreement with the reports of past workers. The pre-oviposition period was 3.49±0.66 days (Chakraborty, 2012)^[3], 3.52±0.18 days (Rai *et al.*, 2003)^[11] and 3.5 days (Solangi *et al.*, 2007)^[16] when reared on *A. gossypii*. More or less similar results were obtained by Shanmugapriya *et al.* (2017)^[13] when reared on *L. erysimi*.

3.5.4 Oviposition period

During ovipositional period, frequent mating was observed in the adults (Fig. 1) and after successful mating the female laid eggs on suitable place. The oviposition period recorded during the study ranged from 15 to 19 days with a mean of 16.40±1.29 days (Table 2). The present findings are consistent with the results of Tank and Korat (2007)^[17] who reported that the oviposition period lasted for 14.38±2.36 days and it was 21.38±2.58 (Chakraborty, 2012)^[3] when reared on *A. gossypii*. Abbas *et al.* (2020)^[1] observed that the oviposition period varied from 10.33-18.33 days when reared on different aphids.

3.5.5 Post-oviposition period

The post-ovipositional period lasted for 1-5 days with a mean of 3.00±0.87 days. More or less similar results were reported by Tank and Korat (2007)^[17] and Chakraborty (2012)^[3] with post-ovipositional period of 3.23±0.72 and 3.22±0.22 days, respectively when reared on *A. Gossypii*. Similarly, Shinde (2012)^[14] reported it as 2.15±0.58 days when reared on *A. craccivora*, while it was 3.36±1.04 on *L. erysimi* (Shanmugapriya *et al.*, 2017)^[13].

3.5.6 Fecundity

The gravid female was able to lay 229-550 eggs with a mean of 363.32±76.32 eggs (Table 2). These results are confirmed with the findings of Tank and Korat (2007)^[17] who reported that the average fecundity of a female when reared on *A.*

Gossypii was 382±163.17 eggs. More or less similar results were reported by Chakraborty (2012)^[3] and Shanmugapriya *et al.* (2017)^[13] with the fecundity of 317.67±12.97 and 323.52±24.80 eggs, respectively.

3.5.7 Adult longevity

The male was able to live upto 14-20 days with a mean of 17.12±1.44 days while the females lived up to 17-28 days with a mean of 22.54±2.38 days (Table 2). This implies that the females were able to live longer than males. These results are in close conformity with the findings of Tank and Korat (2007)^[17] who reported that the longevity of male ranged between 10-21 (mean, 16.09±2.54) days, while in females it was 15-26 (mean, 20.23±2.80) days. A similar trend was followed in the reports of Shanmugapriya *et al.* (2017)^[13] with the average adult longevity in male and female as 16.14±0.38 and 22.56±1.11, respectively. This was further substantiated with the results of Routray *et al.* (2016)^[12], Abbas *et al.* (2020)^[1] and Mishra *et al.* (2021)^[4].

3.6 Total life cycle

The data on the total life span of *C. sexmaculata* on *A. craccivora* were presented in Table 2, and a schematic diagram was depicted in Fig. 1. The present results indicated that the total life span of males varied between 25-38 days with a mean of 29.64±2.59 while in the females it ranged between 30-43 days with a mean of 35.06±3.17 days. The present findings are in close agreement with the results of Tank and Korat (2007)^[17] who mentioned that the mean of the total life span of male and female was 29.72±2.20 and 34.15±2.54 days, respectively. More or less similar to this, Shanmugapriya *et al.* (2017)^[13] noticed that the entire life span of male ranged between 23-41 days (mean, 35.2±3.77), while in females it was 23-43 days (mean, 40.32±3.98). However, the prey quality and environmental conditions have a crucial impact on the biological aspects of Coccinellid beetles (Nyaanga *et al.*, 2012)^[5].

Table 1: Morphometrics of different life stages of *Cheilomenes sexmaculata* on *Aphis craccivora*

Stage	Length (mm)			Breadth (mm)		
	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Egg	0.77	1.12	1.02±0.08	0.34	0.52	0.43±0.04
Larva						
I instar	1.23	1.89	1.58±0.15	0.46	0.79	0.59±0.08
II instar	3.25	4.59	4.01±0.32	0.64	0.96	0.79±0.09
III instar	4.75	6.08	5.41±0.35	1.05	1.26	1.14±0.05
IV instar	6.34	7.36	6.91±0.33	1.29	1.67	1.48±0.09
Pupa	2.92	3.79	3.29±0.24	1.86	2.70	2.21±0.18
Adult						
Male	3.76	4.22	3.96±0.12	3.07	3.35	3.25±0.06
Female	4.76	5.48	5.13±0.19	3.55	4.26	3.98±0.20

Table 2: Life cycle of *Cheilomenes sexmaculata* on *Aphis craccivora* under laboratory conditions

S. No	Particulars	No. observed	Period (Days)		
			Min.	Max.	Mean ± SD
1.	Incubation period (Days)	50	1	3	1.64±0.72
2.	Hatching percentage (%)	50	80	93.94	89.25±3.97
3.	Larval period (Days)				
	I instar	50	1	2	1.26±0.44
	II instar	50	1	3	1.68±0.59
	III instar	50	2	4	2.28±0.54
	IV instar	50	2	4	2.44±0.61
	Total larval period (Days)	50	6	12	7.66±1.61
4.	Pre-pupal (Days)	50	1	2	1.04±0.20

5.	Pupal period (Days)	50	2	4	2.18±0.48
6.	Pre-oviposition period (Days)	25	3	6	3.48±0.77
7.	Oviposition period (Days)	25	15	19	16.40±1.29
8.	Post-oviposition period (Days)	25	1	5	3.00±0.87
9.	Adult emergence (%)	25	81.82	100	89.48±4.44
10.	Sex ratio (Male: Female)	25	1:1.22	1:1.71	1:1.40
11.	Adult longevity (Days)				
	Male	50	14	20	22.54±2.38
	Female	50	17	28	17.12±1.44
12.	Total life cycle (Days)				
	Male	50	25	38	29.64±2.59
	Female	50	30	43	35.06±3.17
13.	Fecundity (No.s)	25	229	550	363.32±76.32

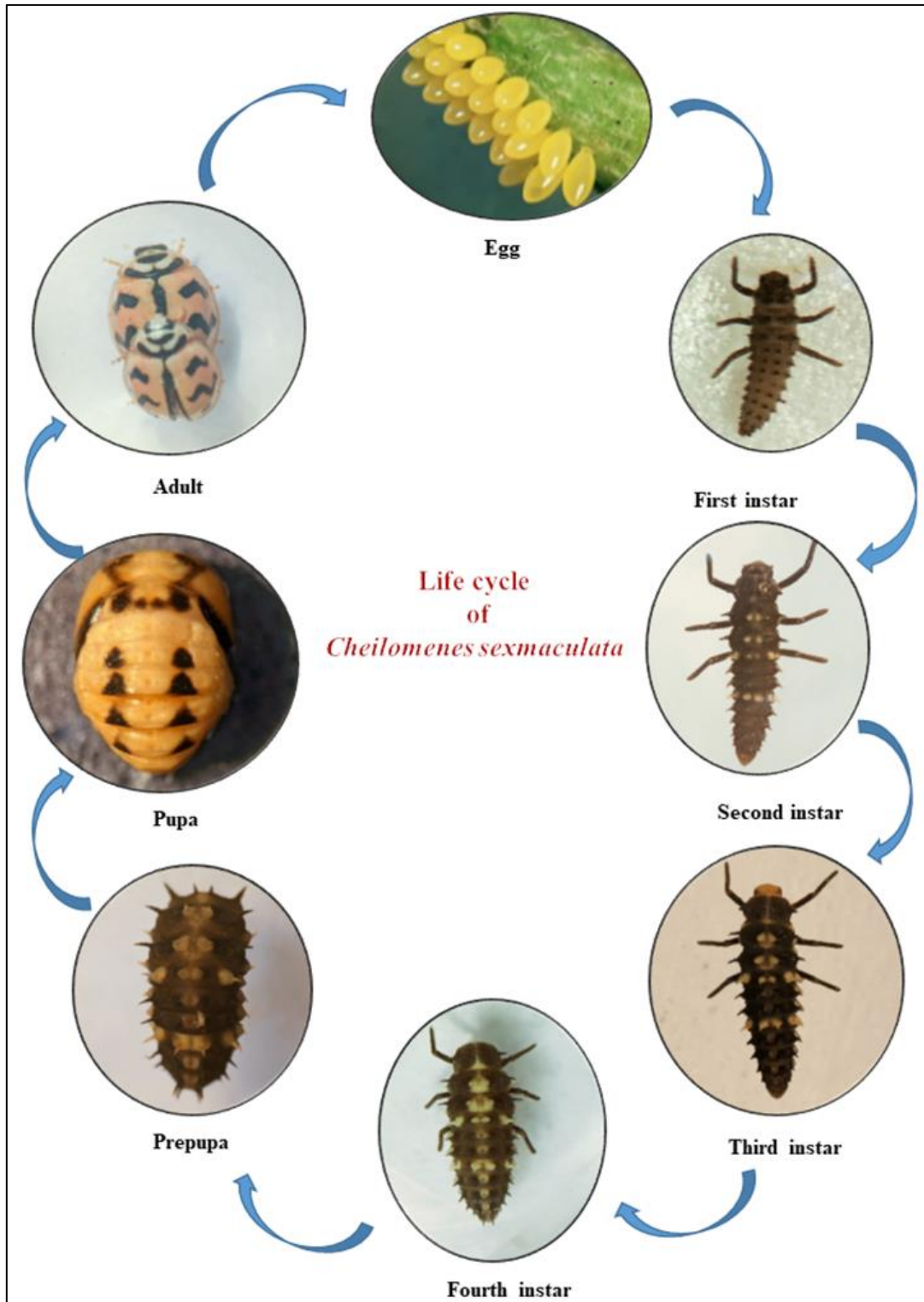


Fig 1: Schematic diagram of life cycle of *Cheilomenes sexmaculata*

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