

## Elytral and Pronotal Polymorphism in the Ladybird beetles (Coleoptera: Coccinellidae) of Hyderabad region.

### Abstract:

The polymorphism of color and pattern variations on the elytra and pronotum of three ladybird beetle species, *Cheilomenessexmaculata* Fabricius, *Hippodamiavariegata* Goeze, and *Propyleadissecta* Mulsant, from the Coccinellidae family in the Hyderabad region of Telangana, India, was investigated during 2019-2020. Through net sweeping and hand picking, adult beetles were collected and identified based on morphological characteristics. A total of four morphs of *C. sexmaculata*, seven of *H. variegata* and eight of *P. dissecta* were observed, indicating significant polymorphic diversity. Interestingly, these morphs were found to intermate freely, posing challenges in species identification, especially with similar-looking species like *Micraspisdiscolor* Fabricius and *Chilocorusnigrita* Fabricius. The abundance of distinct morphs within these populations suggests the influence of selection pressures from both continuous and discontinuous environmental variations.

**Keywords:** Polymorphism, Ladybird beetle, Elytra, Pronotum, Coccinellidae, Coleoptera.

### Introduction:

Polymorphism, characterized by discrete, inherited and alternative phenotypes leading to variability within a population, is a phenomenon ubiquitous across various insect orders such as Lepidoptera, Isoptera, Hymenoptera, Coleoptera, Orthoptera and Odonata (Gullan and Cranston, 2004). This variability can arise from selection acting on continuous or discontinuous variations, resulting in the occurrence of two or more clearly different morphs or forms within a species population, also known as alternative phenotypes. Polymorphism manifests not only between individuals in a population but also between sexes as sexual dimorphism, between geographically separated populations as geographical polymorphism and between generations flying at different seasons of the year as seasonal polymorphism. This diversity confers fitness advantages to organisms in terms of feeding, mating, defending territories and escaping from predators (Singh *et al.*, 2016). Furthermore, camouflage, recognized as a prevalent form of defensive coloration against visually hunting predators (Stevens and Merilaita, 2009), is observed across numerous animal species with contrasting body markings, which, despite their vividness, often render them cryptic against natural backgrounds, particularly to the human eye (Thayer, 1909; Cott, 1940; Endler, 1991; Ruxton *et al.*, 2004).

Among the various types of polymorphism, color polymorphism stands out as one of the most extensively studied examples due to its functional and ecological significance. A notable evolutionary case is observed in the peppered moth, *Bistonbetularia*, where directional color polymorphism within the moth population emerged in response to air pollution during the industrial revolution, resulting in an increased frequency of dark-colored moths - a phenomenon known as industrial melanism (Cook and Saccheri, 2013). Similarly, several species of ladybird beetles, including *Harmonia axyridis*, *Adaliadecempunctata* and *Adaliabipunctata* exhibit elytral color polymorphism, indicating geographical variation (Creed, 1975). However, the underlying reasons for the occurrence of elytral color polymorphism within populations and its variation along geographical gradients remain

incompletely understood. Geographical variations in elytral color polymorphism are often interpreted as adaptations to diverse thermal environments and levels of industrial pollution. For instance, in *Adaliabipunctata*, a reduction in non-melanic types was observed in industrial areas of the UK and Baltic Sea regions, a phenomenon referred to as industrial melanism (Kawakami *et al.*, 2013).

Research on polymorphism in ladybird beetles holds a significant position, as it encompasses variations in color, pattern of markings, number of spots and spot shape on the dorsal surface of the head, elytra and pronotum (Blehman, 2007; Rogers *et al.*, 1971). Changes in abiotic conditions offer an avenue for studying morphs, with melanic morphs often demonstrating better adaptation to cold conditions than non-melanic forms. Consequently, environmental shifts, influencing thermoregulation and predator protection through camouflage, account for differences observed in melanic morphs (Halkka *et al.*, 1975; Thompson, 1984; Capyet *et al.*, 1988 and Singh *et al.*, 2016), thereby elucidating the relationship between various environmental factors and organismal traits. Despite the wealth of literature, scant information exists regarding polymorphism in coccinellid beetles such as *Cheilomenessexmaculata* Fabricius, *Hippodamiavariegata* Goeze and *Propyleadissecta* Mulsant in the Telangana region, with no specific prior records of polymorphism in ladybird beetles. Consequently, the present study aims to investigate polymorphism in ladybird beetles.

### Materials and methods:

Ladybird beetle samples were systematically collected from the campus of Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India, through net sweeping and hand picking at fortnightly intervals during the *Kharif* seasons of 2019 and 2020. Intensive sampling efforts were concentrated within a 300 square meter area covering diverse crops such as brown top millet, finger millet, maize, cotton, bitter gourd, safflower, cabbage, brinjal, coconut and mulberry fields, over a three-month period. Sampling within one square meter plots, replicated five times was conducted to ensure comprehensive coverage. Following collection, beetles were killed using ethyl acetate and subsequently examined in the laboratory for taxonomic identification using stereo-zoom binocular microscopy, with images captured using TCapture software. Specimens were identified to the species level with reference to available literature and male genitalia characteristics as described by Majerus and Kearns (1989). Quantitative analysis of spot variation and qualitative assessment of pronotum and elytra dorsal patterns were utilized to differentiate between morphs within ladybird species.

### Results and discussion:

In this study, the polymorphism of *Cheilomenessexmaculata* Fabricius, *Hippodamiavariegata* Goeze, and *Propyleadissecta* Mulsant was observed in populations of predatory ladybird beetles collected in Hyderabad, Telangana. These species exhibit diverse polymorphic forms, showcasing variations in pronotum and elytral patterns. Certain morphs of *C. sexmaculata* closely resemble external morphologies of *Micraspisdicolor* and *Chilocorusnigrita*, potentially leading to misidentification of the species (Singh *et al.*, 2016). Consequently, besides external characteristics, the shape of male genitalia was utilized for species confirmation. Among the three species, four different morphs of *C. sexmaculata*,

seven different morphs of *H. variegata* and eight different morphs of *P. dissecta* were observed and identified.

### **Polymorphism in *Cheilomenessexmaculata* Fabricius:**

This species has different polymorphic forms, exhibiting variation in the elytral pattern, which freely intermate with each other. Among different specimens collected, four morphs of *C. sexmaculata* were identified based on morphological characters like ground colour, pronotum, spots and pattern of the elytra on the dorsal surface and possessing definite morphology as typical *C. sexmaculata* like length and width of the pronotum and markings on the pronotum (Plate 1).

The description of the important characters of the identified four morphs is as follows:

#### **Morph-I:**

It is a non-melanic kind of morph. The ground colour varied from yellow, light red to orange with black marking on the posterior part of the head, six black patterns on the elytra, including two black zigzag lines and a posterior black spot along with a narrow, black and moderately broad sutural line. A 'T' shaped median marking connecting the broad black band along the posterior margin of the pronotum is present. This is the most prominent form of morph which is commonly called as zig-zag ladybird found throughout the year (Plate 1).

#### **Morph-II:**

This morph represents a non-melanic variation characterized by a yellow ground color on the elytra devoid of any spots, accompanied by a broad black stripe along the suture line. The pronotum exhibits a similar pattern to that observed in morph I. This particular morph was infrequently observed during the month of August, likely due to seasonal variability and prey availability and could potentially be mistaken for species belonging to *Propylea* and *Micraspis* genera (Plate 1).

#### **Morph-III:**

It is a melanic type of morph. The ground colour was black except at margins of the elytra. The posterior sides of both the elytra were having one brownish spot. Pronotum was same as in other forms. The sutural line is indistinct due to the total black colour of the elytra. It is a rare type of morph (Plate 1).

#### **Morph-IV:**

It is a melanic type of morph. The ground colour was black except at margins of the elytra. In the middle of each elytron, a single zig-zag yellow band was present which extended up to scutellum on the anterior side. The sutural line was indistinct due to the total black colour of the elytra. The pronotum remained same as in other forms. It was said to be another rare type of morph (Plate 1).

Kawakami *et al.*, 2013 and 2019 recorded 20 different morphs in *C. sexmaculata* as a result of geographical variation where light type morphs at lower latitudes and dark type morphs at higher latitude were observed and variation might be an adaptation to climatic conditions. Dark morphs may have an advantage in winter due to their higher tolerance to

low temperatures compared to light morphs. Singh *et al.*, 2016 recorded six different morphs of *C. sexmaculata* comprising of three melanic and three non-melanic forms.

Similarities between the present findings and those of previous studies include the recognition of both melanic and non-melanic morphs within *C. sexmaculata* populations. The observed variations could stem from differing geographical regions, environmental pressures, or methodologies employed in sample collection and analysis. Overall, the studies collectively underscore the extensive polymorphism within *C. sexmaculata* populations and highlight the potential role of environmental factors in shaping morphological diversity.

### **Polymorphism in *Hippodamiavariegata* Goeze:**

*H. variegata* has variety of morphs which vary only in margins, size and number of spots on the elytra and pronotum which differed from each other. About seven morphs of this ladybird were recorded in Hyderabad region (Plate 2):

#### **Morph-I:**

The color of the elytra was reddish orange with 13 black spots appearing all over the body of the insect. Each elytron has six black spots and one spot on the scutellum. The first three spots on the top of elytra are arranged in a triangular fashion where the first spot being on the top and in the corner, the second and third spots remaining parallel to each other, fourth and fifth spots are located in the middle third of elytron and sixth spot on posterior end of the elytron. The fourth spot is the largest on the elytron which is oval shaped. Pronotum mostly black with white spots (Plate 2).

#### **Morph-II:**

The color of elytra was reddish orange with nine free black spots and two bands all over the body of the insect. Each elytron has four black spots, one band and one spot on the scutellum. The first three spots on the top of elytra are found in a triangular fashion, the first spot being on the top and in the corner, the second and third spots remaining parallel to each other, fourth spot on posterior end of the elytron and fused band in the middle of the elytra. Pronotum has four black lines apically (Plate 2).

#### **Morph-III:**

The color of elytra is reddish orange with nine black spots appear all over the body of the insect. Each elytron has four black spots and one spot on the scutellum. The first one was on the top of elytra, second and third spots are presented in the middle third of elytron and fourth spot on posterior end of the elytron. Pronotum was mostly black with white spots (Plate 2).

#### **Morph-IV:**

The color of the elytra was reddish orange with nine free black spots and two bands all over the body of the insect. Each elytron has four black spots, one band and one spot on scutellum. The first three spots on the top of elytra were found in a triangular fashion, the first spot being on the top and in the corner, the second and third spots parallel to each other, fourth spot on posterior end of the elytron and fused band in the middle of the elytra. The anterior three spots were smaller in size and pronotum was mostly black with white spots (Plate 2).

### **Morph-V:**

The color of elytra is reddish orange with three black spots and four lightly fused bands all over the body. Each elytron has one black spot on posterior end of the elytron, one spot on the scutellum, one band in the anterior end of the elytra formed by the fusion of the three triangular spots and one band in the mid of the elytra formed by the fusion of two spots. Pronotum is with four black lines apically (Plate 2).

### **Morph-VI:**

Pronotum was mostly black with white spots. Color of elytra was reddish orange with two black spots and four thickly fused bands all over the body of the insect. Each elytron has one black spot on the posterior end, one band in the anterior end of the elytra formed by the fusion of the three triangular spots along with the spot on the scutellum and one band in the mid of the elytra formed by the fusion of two spots (Plate 2).

### **Morph-VII:**

Pronotum was mostly black with white spots. The color of elytra was reddish orange with two 'C' shaped and two incomplete 'W' shaped bands without any spots on the elytra. Each elytron has one incomplete 'W' shaped band in the anterior end formed by the fusion of the three triangular spots along with the spot on scutellum and one 'C' shaped band on lower half of the elytron (Plate 2).

Honeket *et al.*, 2012 reported 22 different morphs of *H. variegata* in the middle part of Europe and Slovakia. Similarly, Zareet *et al.*, 2013 reported four different morphs and Biranvand and Shakarami, 2014 reported 18 different morphs of the species based on morphological characteristics from Central region of Iran.

Similarities can be noted between the observed morphs and those reported in the cited studies (Honeket *et al.*, 2012; Zareet *et al.*, 2013; Biranvand&Shakarami, 2014) in terms of the number of morphs identified and certain morphological characteristics. Additionally, the presence of bands and spots on the elytra, as well as variations in pronotum coloration, were common features across all studies, suggesting consistency in morphological diversity within the species.

However, differences also emerge, particularly regarding the specific patterns and arrangements of bands and spots on the elytra. While some morphs observed in the present study exhibit unique patterns such as 'C' and 'W' shapes, other studies may report different configurations or a wider array of patterns. Additionally, variations in the number and arrangement of spots and bands may differ between studies, reflecting potential geographic or environmental influences on morphological traits within *H. variegata* populations.

Overall, while there are notable similarities in the general patterns of polymorphism observed in *H. variegata* across different studies, differences in specific morphological characteristics highlight the complexity and variability inherent in ladybird beetle populations across various regions and habitats.

### **Polymorphism in *Propyleadissecta***

*Propyleadissecta* is a polymorphic species with typical (three black streaks on elytra), intermediate (pale black elytral markings) and pale (scarlet without black elytral markings)

morphs. Among the different specimens collected, eight morphs of *Propyleadissecta* were identified based on morphological characters like ground colour, pronotum, spots and pattern of the elytra and pronotum on the dorsal surface (Plate 3).

The description of the important characters of the identified eight morphs is as follows:

**Morph-I:**

Elytra is yellowish orange and spotless all over the body of the insect. Pronotum has black patch restricted to the mid-dorsal distal end with creamy white pronotum having wavy margin along its proximal end (Plate 3).

**Morph-II:**

Elytra is yellowish orange and spotless all over the body of the insect. Pronotum has a black patch extending all along mid-dorsal line from proximal to distal end with a bulging appearance towards the distal end of the pronotum (Plate 3).

**Morph-III:**

Elytra is yellowish orange with two pale yellow regions adjoining the elytral sutural line and two black spots on the anterior margin of the elytra. Pronotum has black patch restricted to the mid-dorsal distal end of the creamy white pronotum with a wavy margin along its proximal end (Plate 3).

**Morph-IV:**

Elytra is yellowish orange with two black spots on the posterior margin. Pronotum has black patch restricted to the mid-dorsal distal end of the creamy white pronotum with a wavy margin along its proximal end (Plate 3).

**Morph-V:**

Elytra is yellowish orange with three black streaks all over the body of the insect. Pronotum has a black patch extending all along mid-dorsal line from proximal to distal end with a bulging appearance towards distal end of the pronotum (Plate 3).

**Morph-VI:**

Elytra is yellowish orange with three black streaks all over the body. Pronotum has black patch restricted to the mid-dorsal distal end of the creamy white pronotum with a wavy margin along the proximal end (Plate 3).

**Morph-VII:**

Elytra is yellowish orange with two black spots anteriorly and two black spots posteriorly. Pronotum has a black patch extending all along mid-dorsal line from proximal to distal end with a bulged appearance towards the distal end of the pronotum (Plate 3).

**Morph-VIII:**

Elytra is yellowish orange with two pale yellow regions adjoining the elytral sutural line with two black spots anteriorly and two black spots posteriorly. Pronotum has a black patch

extending all along mid-dorsal line from proximal to distal end with bulged appearance towards the distal end of the pronotum (Plate 3).

### **Conclusion:**

The present study was an attempt made to document polymorphism among the ladybird beetles i.e., *C.sexmaculata*, *H.variegata* and *P.dissecta* in the Hyderabad region, Telangana, India. The results of this study revealed four morphs of *C. sexmaculata*, seven morphs of *H. variegata* and eight morphs of *P. dissecta* which helped to explore, identify and prepare inventory of ladybird beetles morphs in Hyderabad region. Species of these ladybird beetles showed high variability with respect to pattern of the elytra and the pronotum. Different morphs of the ladybird beetles have differences in the appearance of number of spots, size, and distribution pattern on the elytra and the pronotum. Among the four different morphs of *C. sexmaculata*, morph-I is the most prevalent and found throughout the year as the most successful one in the studied area. The difference in the reproductive success in the various morphs in polymorphism, lead to the success of one or two most suited morphs, in comparison to the other morphs giving the fittest an adaptive advantage in the selection. The darker morphs were found during the cooler season which help to absorb more light and the lighter morphs reduce the heat stress in hotter seasons. These morphs may create confusion in identification on external basis, so for more confirmation, study of the male genitalia is essential.

Variability among these morphs may be the outcome of their genetic constitution, or differences in the environment to which they were exposed, or the combination of both. Based on this study, it may be concluded that these ladybird beetles show variable elytral and pronotal polymorphism with varied number of morphs in Hyderabad region. The findings of this study will certainly update the present knowledge of ladybird beetles polymorphism in Telangana, India.

### **COMPLIANCE WITH ETHICAL STANDARDS:**

Conflict of interest: The authors declare that they have no conflict of interest.



Morph-I

Morph-II

Morph-III

Morph-IV

**Plate 1: Different morphs of *Cheilomenessexmaculata***



UNDER PEER REVIEW

Morph-I                      Morph-II                      Morph-III                      Morph-IV                      Morph-V





Morph-VI	Morph-VII
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**Plate 2: Different morphs of *Hippodamiavariegata***

UNDER PEER REVIEW

Morph-I

Morph-II

Morph-III

Morph-IV

Morph-V





Morph-VI

Morph-VII

Morph-VIII



**Plate 3: Different morphs of *Propyleadissecta***

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