

Elytral and Pronotal Polymorphism in the Ladybird beetles (Coleoptera: Coccinellidae).

Abstract:

Polymorphism with respect to variation in elytra and pronotum in *Cheilomenes sexmaculata* Fabricius, *Hippodamia variegata* Goeze and *Propylea dissecta* Mulsant of the family, Coccinellidae, order, Coleoptera was studied during 2019-2020 in Hyderabad region (Telangana, India). Adults collected by net sweeping and hand picking were identified based on their morphological characters. A total of four different morphs of *C. sexmaculata*, seven different morphs of *H. variegata* and eight different morphs of *P. dissecta* were observed and identified. These species have different polymorphic forms, exhibiting variation in the elytral and pronotum patterns, which could freely intermate with each other, as some of the morphs were similar in their external morphology with *Micraspis discolor* Fabricius and *Chilocorus nigrita* Fabricius which could lead to misidentification of the species. Occurrence of these many morphs of ladybird beetles with different morphological characteristics within a population might be due to the selection pressure over continuous or discontinuous variations.

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

Introduction:

Polymorphism always refers to the variability within a population. Polymorphism is the occurrence of two or more clearly different morphs or forms in the population of a species, also referred to as alternative phenotypes. Many types of polymorphism can be seen in the insect order Lepidoptera, Isoptera, Hymenoptera, Coleoptera, Orthoptera, Odonata etc (Gullan and Cranston, 2004) which was due to selection over continuous or discontinuous variations. Polymorphism can be seen not only between individuals in a population, but also between the sexes as sexual dimorphism, between geographically separated populations as geographical polymorphism and also between generations flying at different seasons of the year as seasonal polymorphism. Polymorphism gives a fitness advantage to the organisms in terms of feeding, mating, defending territories and escaping from predators (Singh *et al.*, 2016).

Among different types of polymorphism, colour polymorphism provides one of the best characterized examples of functionally and ecologically important polymorphisms. Many species of ladybird beetles, i.e., *Harmonia axyridis*, *Adalia decempunctata* and *Adalia bipunctata* show elytral colour polymorphism, which indicates geographical variation (Creed, 1975). In spite of that why elytral colour polymorphism occurs in a population and why it varies along geographical gradients have not been fully understood. Geographical variation in elytral colour polymorphism has been interpreted as an adaptation to varying thermal environments and industrially polluted air. In *Adalia bipunctata* there was reduction of non-melanistic types in the industrial areas of UK and Baltic Sea areas (Kawakami *et al.*, 2013) which was mentioned as industrial melanism.

Studies on polymorphism of ladybird beetles have occupied a special position which is related to pattern of the dorsal surface of the head, elytra and pronotum (Blehman, 2007; Rogers *et al.*, 1971). The changes in the abiotic conditions provide an opportunity for the

Comment [SD1]: Is the polymorphism strictly related to color and pattern variations on the elytra and pronotum, or are there other traits involved?

If genetic mechanisms are implied, are they explicitly mentioned or supported by evidence?

Comment [SD2]: Is the statement about selection pressure based on specific data or hypotheses from the study? Are alternative explanations considered?

Comment [SD3]: How does this research contribute to understanding polymorphism in ladybird beetles or ecological variation in general?

Comment [SD4]: While the initial definition mentions variability, it could be strengthened by explicitly stating that polymorphism refers to discrete, inherited, and alternative phenotypes within a population. This distinguishes it from continuous variation.

Comment [SD5]: needs clarification. Selection acts on existing variation, not causes it. Consider rephrasing as "can arise from selection acting on..."

Comment [SD6]: The claim about fitness benefits could be expanded upon. Mention specific examples of how different morphs might have advantages in different contexts (e.g., camouflage, thermoregulation).

Comment [SD7]: The list of insect orders is extensive. Consider mentioning 1-2 prominent examples with well-characterized polymorphism (e.g., peppered moths) for clarity

Comment [SD8]: While the importance of dorsal patterns is acknowledged, clarify how the study specifically addresses these patterns in relation to polymorphism.

study of morphs, leading the melanic morphs adapting better to cold conditions than the non-melanic forms. Therefore, the environmental changes and protection from predators explain the difference in the melanic morphs (Halkka *et al.*, 1975; Thompson, 1984; Capy *et al.*, 1988; Singh *et al.*, 2016). As a result, polymorphism may explain the relation between different environmental factors and characters of the living organisms. The study of available literature revealed scanty information related to polymorphism in the coccinellid beetles viz., *Cheilomenes sexmaculata* Fabricius, *Hippodamia variegata* Goeze and *Propylea dissecta* Mulsant in Telangana region, with no specific previous record of polymorphism in ladybird beetles. Therefore, the present study intends to find polymorphism in ladybird beetles.

Comment [SD9]: Discuss the potential selective pressures favoring melanic morphs beyond winter tolerance. Consider camouflage, predation, or thermoregulation as alternatives.

Materials and methods:

Ladybird beetle samples were collected from the college campus of Professor Jayashankar Telangana State Agricultural University, Hyderabad, Telangana, India, by net sweeping and hand picking at fortnight intervals during *Kharif* 2019-2020. Intensive collections were made in brown top millet, finger millet, maize, cotton, bitter gourd, safflower, cabbage, brinjal, coconut and mulberry fields. After collection, the beetles killed and were observed in the laboratory for various taxonomic characters under stereo-zoom binocular microscope and photographed using TCCapture software. Specimens were identified to species level with the help of available literature and characteristics of male genitalia. The variation in the spots and pattern of pronotum and elytra on the dorsal surface was used for differentiating different morphs of the ladybird species.

Comment [SD10]: Briefly mention the specific aspects of polymorphism lacking information in the chosen species. This will better connect the literature review to the study's objectives.

Instead of simply stating the intent to find polymorphism, clearly articulate the research questions or specific aspects of polymorphism the study aims to address.

Comment [SD11]: Mention the total number of samples collected and whether the sampling effort was consistent across different habitats.

If feasible, consider including a map or description of the sampling locations within the college campus.

Comment [SD12]: Mention the specific method used to kill the beetles and ensure it adheres to ethical considerations.

Results and discussion:

In this study, the polymorphism of *Cheilomenes sexmaculata* Fabricius, *Hippodamia variegata* Goeze and *Propylea dissecta* Mulsant was observed in the populations of predatory ladybird beetles collected at Hyderabad, Telangana feeding on aphids, leafhoppers, whiteflies, mealybugs, and scales. These species have different polymorphic forms, showing variation in pronotum and elytral pattern. Some of the morphs of *Cheilomenes sexmaculata* were very similar in their external morphology with *Micraspis discolor* and *Chilocorus nigritus* which could lead to misidentification of the species (Singh *et al.*, 2016). As the result, apart from external characters, the shape of the male genitalia was used to confirm the species. 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.

Comment [SD13]: Male genitalia were examined for species identification, briefly mention how this was done and whether it contributed to morph differentiation.

Comment [SD14]: Clarify how variation in spots and patterns was used to define and differentiate morphs. Were there quantitative (e.g., number of spots) or qualitative (e.g., shape of patterns) criteria used?

Comment [SD15]: It's not necessary to repeat the known prey preferences here. Instead, focus on whether feeding habits were linked to specific morphs or if prey availability influenced polymorphism.

Polymorphism in *Cheilomenes sexmaculata* Fabricius:

This species has different polymorphic forms, exhibiting variation in the elytral pattern, which could 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).

Comment [SD16]: Explore the broader ecological impacts of polymorphism in *C. sexmaculata*. How might it affect prey consumption, predator avoidance, or population dynamics?

Comment [SD17]: Avoid statements like "could freely intermate" without evidence. Discuss known factors impacting interbreeding between morphs or geographically separated populations.

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

Comment [SD18]: Report the abundance or frequency of each morph observed. Are they equally distributed or is there a dominant form?

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:

It is a non-melanic kind of morph. The ground colour was yellow without any spots on the elytra with a broad black stripe as suture line and pronotum was of same type as seen in the morph I. This type of morph was seen rarely in the month of August and could be easily confused with *Propylea* and *Micraspis* species (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.

Polymorphism in *Hippodamia variegata* 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).

Comment [SD19]: Instead of subjective terms like "broad" or "narrow," use quantitative measurements to describe spots, patterns, and markings. Consider including a standardized color reference system.

Use consistent terminology (e.g., elytra vs. wing covers) throughout the text.

Comment [SD20]: Elaborate on the significance of observing a specific morph only in August. Is it related to seasonal changes in prey availability or temperature?

Comment [SD21]: Compare your findings with those of Kawakami *et al.* (2013, 2019) and Singh *et al.* (2016) in greater detail. Highlight similarities, differences, and potential explanations.

Comment [SD22]: Report the abundance or frequency of each morph observed. Are they evenly distributed, or is there a dominant form?

Comment [SD23]: Combine descriptions of similar morphs (e.g., Morph-III and Morph-IV) for conciseness.

Comment [SD24]: Use precise measurements (e.g., millimeter, number of pixels) instead of subjective terms like "large" or "small" for spots and other features.

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).

Honek *et al.*, 2012 reported 22 different morphs of *H. variegata* in the middle part of Europe and Slovakia. Similarly, Zare *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.

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:

Comment [SD25]: Highlight similarities and differences between your observed morphs and those reported in cited studies (Honek et al., 2012; Zare et al., 2013; Biranvand & Shakarami, 2014).

Comment [SD26]: Explain how your eight morphs relate to the "typical," "intermediate," and "pale" morphs mentioned initially. Are they subcategories?

Comment [SD27]: The descriptions for Morph-V and Morph-VI seem identical. Verify if this is correct. It's unclear how the elytral "regions" in Morph-VIII differ from the spots in other morphs. Clarify this description.

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.

Comment [SD28]: While mentioning seasonal variation in morph abundance, provide evidence or references supporting the link between morph color and temperature tolerance. Discuss alternative explanations for morph frequency changes.

Comment [SD29]: Acknowledge the complexity of polymorphism causes and avoid simplifying it as "genetic constitution" or "environment." Discuss the potentially interacting roles of genetics, environment, and their interactions.

Comment [SD30]: Acknowledge any limitations of the study (e.g., sample size, seasonality) and suggest future research directions (e.g., genetic analysis, fitness studies, predator-prey interactions).



Morph-I

Morph-II

Morph-III

Morph-IV

Plate 1: Different morphs of *Cheilomenessexmaculata*



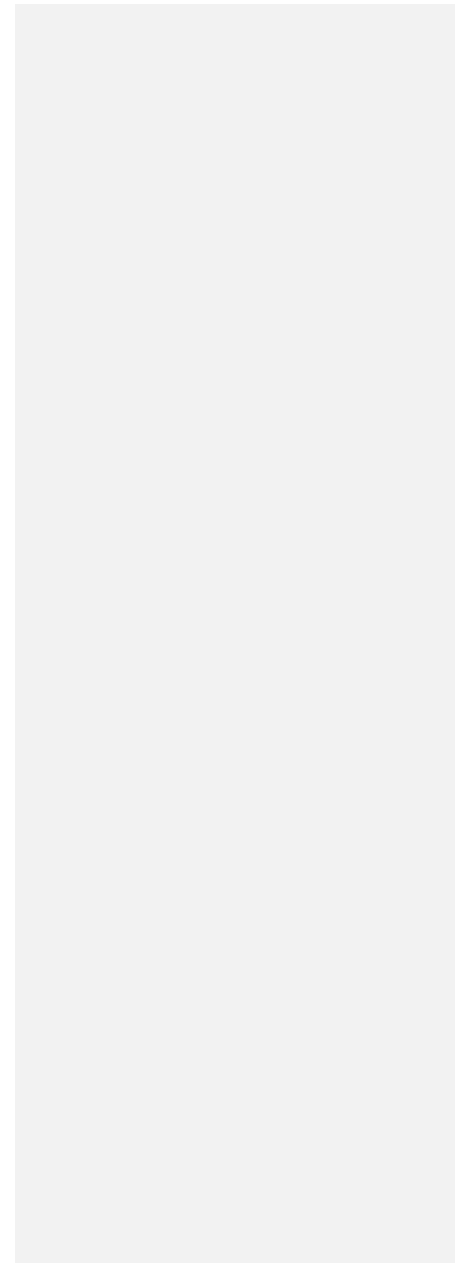
Morph-I

Morph-II

Morph-III

Morph-IV

Morph-V



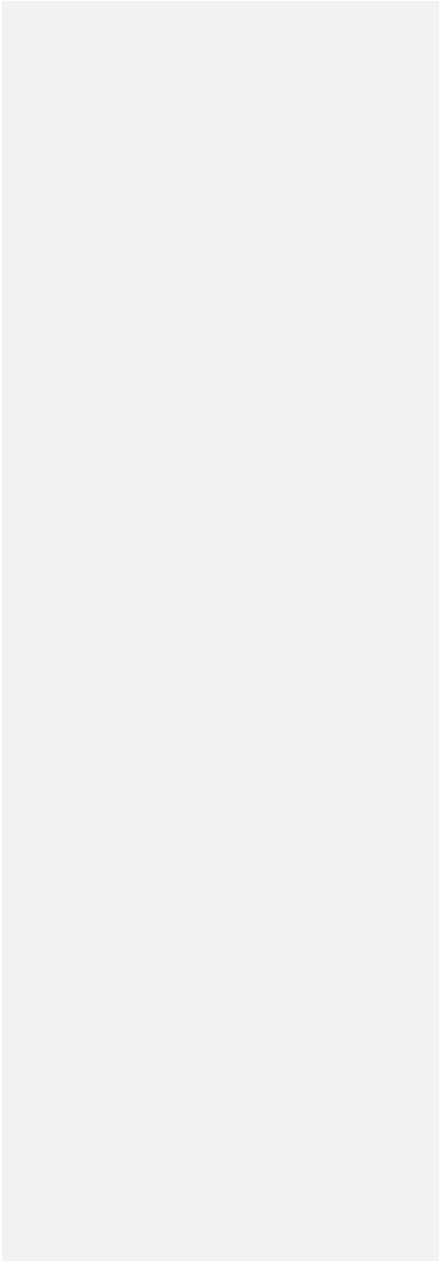


| | |
|----------|-----------|
| Morph-VI | Morph-VII |
|----------|-----------|



Plate 2: Different morphs of *Hippodamia variegata*

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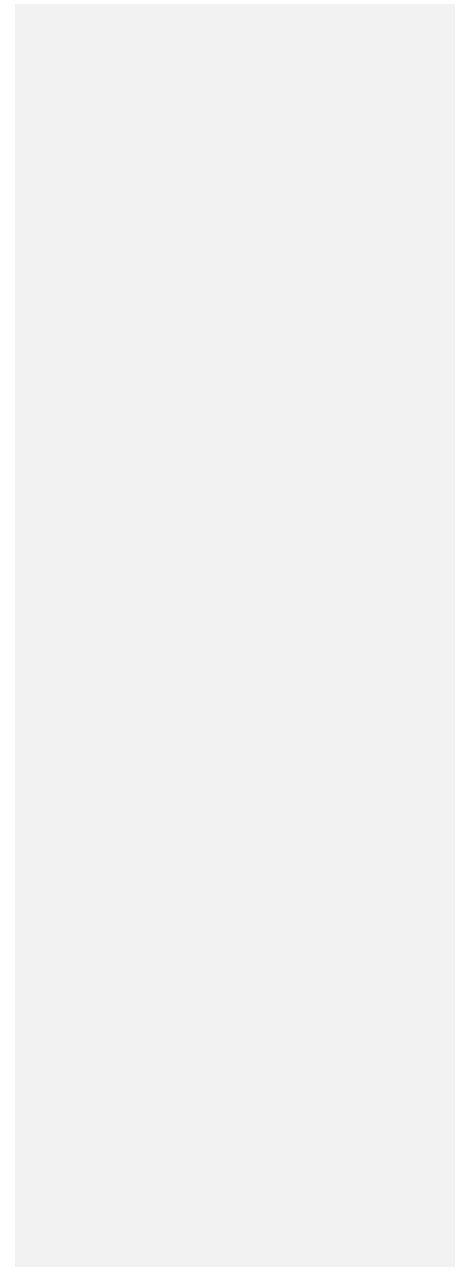
Morph-I

Morph-II

Morph-III

Morph-IV

Morph-V





Morph-VI

Morph-VII

Morph-VIII



Plate 3: Different morphs of *Propyleadissecta*

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Comment [SD31]: Include journal volume number after issue number (e.g., 5(2), 25-36).

Comment [SD32]: Include page range after journal volume and issue number (e.g., 61, 263-268).

Comment [SD33]: Ensure consistency in formatting author initials (use space after initials and period after each initial).

Comment [SD34]: Remove additional comma after "K., M.".

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