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Exploring the diversity of coccinellid species: A comprehensive study on polymorphism and species-specific male genitalia identification

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ABSTRACT: In this study, the diversity of coccinellids along with their polymorphic forms is presented, by studying the structure of male genitalia and siphon. Out of the fourteen species of coccinellids listed, eleven species were predaceous on aphids. The male genitalia structures of the five most common species i.e., *Coccinella septempunctata*, *Co. transversalis*, *Cheilomenes sexmaculata*, *Micraspis discolor* and *M. yasumatsui* have been described and found to exhibit characteristic differentiating features. The melanic and non-melanic forms of *Ch. sexmaculata*, the most ubiquitous coccinellid in the Indian sub-continent, were dissected and shown to have similar male genitalia structures. Problem of misidentification due to polymorphic forms of coccinellid beetles can be mitigated by studying male genitalia and siphonal structures in addition to external morphological studies. © 2024 Association for Advancement of Entomology

KEY WORDS: Ladybird beetles, different morphs, siphonal structures

INTRODUCTION

In agricultural and horticultural crop ecosystems, coccinellids are crucial group of predators preserved and augmented to achieve both conservation and applied biological pest control. These beetles are economically valuable due to their predation on wide range of crop pests, primarily homopterans, including aphids, coccids, and other soft-bodied insects, in both their larval and adult stages (Hippa *et al*., 1978; Kring *et al*., 1985). In general, the horticultural crop ecosystems in both regions were found to have a higher richness of coccinellid species compared to agricultural crop ecosystems (Gurung *et al*., 2019). Understanding of coccinellid biodiversity enhances our knowledge of predator behaviour in relation to pest reproduction, population dynamics, and crop impact, which is critical for developing effective pest management strategies. The presence of different morphs within the same species—such as morphological variation in the elytral colour—can complicate species identification (Gullan and Cranston 2014).

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In most insect families, the complex genitalia, which are connected to the distal segments of the abdomen, serve as the primary basis for species differentiation and aid in family identification (Dobzhanskiy, 1926). Since the male genitalia of a species are unique, morphological analysis of these structures is one of the most critical factors in taxonomic identification below the family level (Chowdary *et al.,* 2015). Unlike female genitalia, where species-specific traits are less pronounced, male genitalia are typically heavily sclerotized, rigid structures that exhibit greater variability and are often the primary focus in studies of genital evolution (Richmond *et al*., 2016). Elytral colour polymorphism is known to occur in many ladybird species, and understanding this polymorphism is crucial for accurate species identification. Since elytral colour polymorphism in coccinellids can lead to misidentification based on external morphological characters, further confirmation through the study of male genitalia is essential.

MATERIALS AND METHODS

The study was carried out at the Instructional Farm of the Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, in Pundibari, Cooch Behar, West Bengal, India. The farm is situated at $26^0$19’86"N latitude and $89^0$23’53"E longitude, with an altitude of 43m above mean sea level. The region falls within the Terai agroclimatic zone of West Bengal. The morpho-taxonomical studies of coccinellids were conducted in the Entomology lab at the Regional Research Station (Terai Zone), Directorate of Research, UBKV. Coccinellid beetle adults were randomly collected using a sweep net and by hand-picking. The specimens were dried in a hot air oven at 45–50°C for 4-6 hours after being killed with ethyl acetate in killing bottles. The collected specimens were examined using a ZEISS Stemi 508 stereo microscope (8:1 zoom) fitted with ZEISS Axiocam 105 colour camera and Carl Zeiss Zen 2.5 lite (blue edition) imaging software. In some cases, images were processed into composite images for enhanced depth and clarity using Combine ZP and edited with Microsoft Paint 3D. The coccinellids were identified at the Entomology lab, RRS (Terai Zone), using previously preserved specimens identified by an expert systematist, along with reference to literature and checklists (Poorani, 2002). Later, Dr. J. Poorani of NRC Banana, Trichy, identified and confirmed all the coccinellid specimens collected from various crop ecosystems of the UBKV campus.

Dissections were performed on the collected coccinellid beetles under a microscope to determine their sexes. The specimens were delicately positioned on a dissection tray, placed on their backs for dissection of both male and female genitalia. Using microneedles and applying gentle pressure at the thorax-abdomen intersection, the abdomen was separated from the thoracic region under a binocular microscope. To aid in the digestion of soft tissues, the separated abdomen was transferred to cavity blocks containing a few millilitres of freshly prepared 10 per cent KOH, using a camel hair brush. These cavity blocks were then left at room temperature overnight. The following day, the abdomen was carefully removed from the KOH solution using blunt needles and placed in a glass hollow dish containing distilled water. Soft tissues that had been digested were gently squeezed out. The abdomen was then placed on a glass slide with one or two drops of glycerine for genitalia dissection, after being repeatedly rinsed in distilled water. This process rendered the entire abdomen transparent, facilitating the examination of the genitalia.

The male genitalia consist of a tegmen and a sipho (Fig. 1). The tegmen comprises a basal piece, a distinct median projection or basal lobe (often incorrectly referred to as the median lobe), a pair of lateral arms or parameres, and a median basal strut or trabes. In some papers, the tegmen is collectively referred to as the aedeagus. The median lobe may be symmetrical or asymmetrical (as in the tribe Serangiini). The parameres are typically setose at their apices and / or along the outer margins. The sipho is usually elongated, tubular, and curved, with a basal siphonal capsule and various modification at the apex. It functions as the true penis or the intromittent organ. The structure of the male genitalia is species-specific and serves as the most important diagnostic feature, particularly in the absence of other reliable external diagnostic characters (Poorani, 2022).

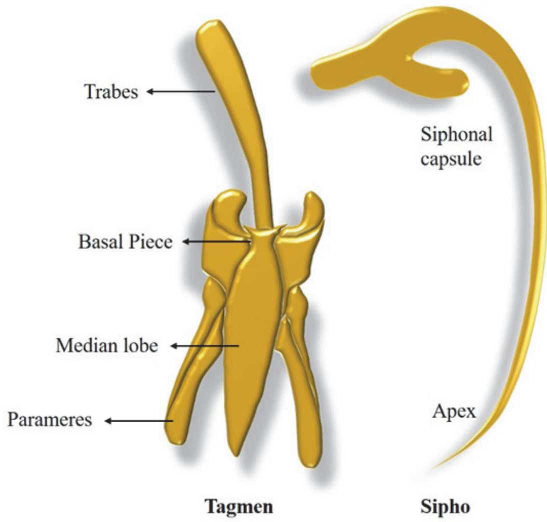

Fig.1: Structure of male genitalia

RESULTS AND DISCUSSION

Biodiversity and Identification based on external morphology of coccinellids: In the present investigation on the biodiversity and diagnostic characteristics of coccinellids collected from the sub-Himalayan Terai region of West Bengal, fourteen species belonging to nine genera and three tribes under the subfamily Coccinellinae of the family Coccinellidae were documented (Fig.2).

Sub family: Coccinellinae; Tribe: Sticholotidini (Pharini)

1. *Sticholotis* sp.

The body is slightly elongated with a glabrous outer surface. The head, pronotum, and scutellum are brownish-yellow. The elytra are also brownish-yellow, each with five black spots, for a total of ten spots across both elytra.

Tribe : Coccinellini

2. *Coccinella septempunctata* Linnaeus

The body is slightly elongated, with a black head and scutellum. The pronotum is black with orange-yellow coloration on the anterolateral sides. The elytra are yellowish-brown to reddish-brown, featuring a total of seven black spots: three on each elytron and one median black spot located on the mid-dorsal line at the junction of the elytra.

3. *Co. transversalis* Fabricius

The body is slightly elongated, with the head and scutellum black. The pronotum is black, with orange coloration on the anterolateral sides. The elytra are dull orange to yellowish-brown, featuring six stripes—three on each elytron.

4. *Harmonia octomaculata* (Fabricius)

The body is slightly elongated, with the head, pronotum, and scutellum black. The elytra are yellowish-brown, each featuring one stripe.

5. *H. dimidiata* (Fabricius)

The pronotum is orange-yellow to bright red with a pair of black spots, which are often fused into a single marking with a median emargination. The elytra are orange-yellow to bright red, featuring 13 black spots arranged across their surface.

6. *Micraspis discolor* (Fabricius)

The elytra are oval, flat underneath, convex in shape, and red in colour. The margins of the elytra form a joint median black streak along the mid-dorsal line.

7. *M. yasumatsui* Sasaji

The head, pronotum, and scutellum is light brown. The elytra are dark red and lack any spots, with no deviations along the mid-dorsal line.

8. *Cheilomenes sexmaculata* (Fabricius)

The body is oval in shape, moderately convex from the dorsal side. The elytra feature six black macular spots, including two zigzag lines. The hind pair of wings, abdomen, and eyes are yellow in colour.

9. *Propylea dissecta* (Mulsant)

The body is slightly elongated. The head is brown, the scutellum is black, and the pronotum is black

Fig. 2 a. *Coccinella septempunctata,* b. *Micraspis discolor,* c. *Illeis indica*, d. *Harmonia dimidiata,* e. *Micraspis yasumatsui,* f. *Coccinella transversalis* g. *Sticholotis* sp., h. *Synonychimorpha chittagongi,* i. *Cheilomenes sexmaculata,* j. *Harmonia octomaculata,* k. *Propylea dissecta*, l. *Henosepilachna vigintioctopunctata,* m. *Henosepilachna septima,* n. *Henosepilachna pusillanima*

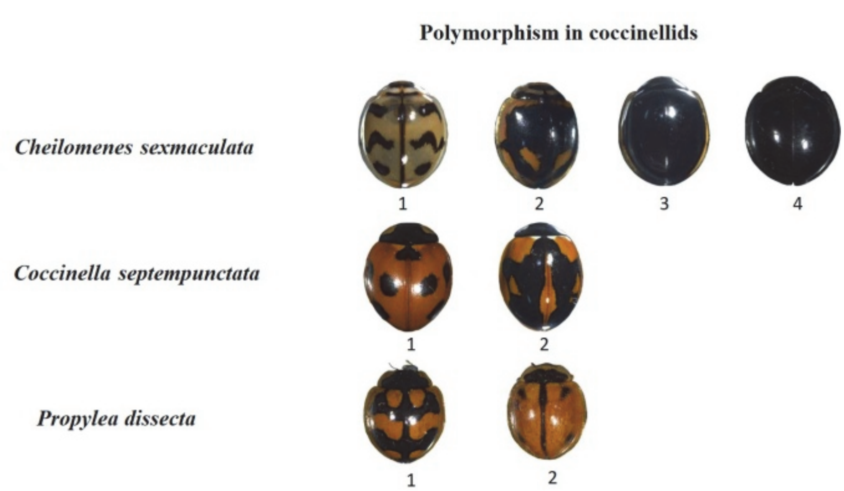

Fig. 3 Polymorphism in coccinellids found in Terai region

with half of it being pale yellow. The elytra are brownish with four black spots, two on each elytron.

10. *Illeis indica* Timberlake

The head is white, and the scutellum is black. The pronotum is pale yellow with two black spots. The elytra are creamy yellow, without any spots or stripes.

11. *Synonychimorpha chittagongi* (Vazirani)

The body is tiny and round or oval. The head, scutellum, and pronotum are yellow. The elytra are black with a broad yellow border along the outer margin, and they lack any spots or stripes.

Tribe : Epilachnini

12. *Henosepilachna vigintioctopunctata* (Fabricius)

The body is slightly elongated. The head, pronotum, and scutellum is deep red to orange. The elytra are deep red to orange with 7-14 black spots on each elytron. The anterior margin of the elytra, where it meets the pronotum, is not truncated, and the tips of the elytra are rounded.

13. *H. septima* (Dieke) Syn. *Epilachna demurili*

The body is moderately elongated. The head, pronotum, and scutellum are light copper-coloured and dull in appearance. The elytra are light copper-coloured with six black spots on each elytron. The anterior margin of the elytra, where it meets the pronotum, is truncated, and the tips of the elytra are somewhat pointed.

14. *H. pusillanima* (Mulsant) Syn. *Epilachna dodecastigma* (Wiedemann)

The body is moderately elongated. The head, pronotum, and scutellum are deep copper-coloured. The elytra are also deep copper-coloured, with six black spots on each elytron. The anterior margin of the elytra, where it meets the pronotum, is truncated, and the tips of the elytra are more rounded compared to *H. septima*.

Host plants of phytophagous coccinellids under Terai zone of West Bengal: *Henosepilachna vigintioctopunctata* and *H. pusillanima* attack various solanaceous vegetables, such as brinjal, tomato, and potato, as well as solanaceous weeds. However, in the population collected from UBKV, Pundibari, the majority individuals were *H. vigintioctopunctata* (confirmed from the specimens sent to Dr. J. Poorani for identification, which is also supported by available literature). *H. septima* exclusively feeds on cucurbitaceous vegetables and weeds.

Polymorphism in coccinellids: During the biodiversity study of the coccinellids, different morphological variations were observed in some common species of ladybird beetles. Four morphs of *C. sexmaculata* and two morphs each of *Coccinella septempunctata* and *Propylea dissecta* were identified (Fig. 3).

Among the four morphs of *C. sexmaculata*, two morphs (morph 1 and 2) are non-melanic, distinguished by physical characteristics such as ground colour, pronotum, spots, and the elytral pattern on the dorsal surface. The other two morphs (morph 3 and 4) are melanic and can only be identified by examining the male genitalia. The appearance of the male genitalia served as the primary method for confirming the species of all morphotypes. Upon examining the male genitalia, particularly sipho, of one non-melanic form (morph 1) and one melanic form (morph 4), the same siphonal structure was observed (Fig. 4).

In this way, in addition to studying external morphological characteristics, the shapes and structures of the male genitalia, particularly sipho were essential for accurately identifying the species and addressing the problem of misidentification among the morphotypes of the same species.

Male genitalia structure of common coccinellid species recorded at Pundibari:

Coccinella septempunctata (Fig. 5)

Sipho: The siphonal tube is long, bent at base, almost straight for most of its length, and the distal end carries more or less sac-like structure. The apex appears to be distorted at three points. The siphonal capsule is bloated and dense.

Tegmen: The trabes are short and more or less uniform in thickness. The Median lobe is very broad at base, tapering gradually beyond the middle to the apex, forming a triangle-like structure. The Parameres are comparatively shorter than median lobe, covered with dense long hairs on the dorsal side except at the base.

Coccinella transversalis (Fig. 6)

Sipho: The siphonal tube is comparatively short, curved at the base, and pointed. The siphonal tube carries a transparent, bubble-like structure on the dorsal side of the sub distal portion.

Tegmen: The trabes are comparatively long, flat, and thick; the basal piece is quadrate. The median lobe is broad apically, narrowing towards the tip, and longer than the parameres. It is deeply emarginated in the distal half of its length, with the tip extending to form a tongue-like structure.

Cheilomenes sexmaculata (Fig. 7)

Sipho: The siphonal tube is strongly curved at the base; the siphonal capsule is well-developed, with the outer arm longer and pointed and inner arm shorter and rounded. The siphonal apex is thread-like.

Tegmen: The trabes are longer and straight; the median lobe is shorter than the parameres; the parameres are cylindrical and slightly bent at the apical end.

Micraspis discolor (Fig. 8)

Sipho: The siphonal tube is strongly curved at the base and up to half of its length, and straight at the apex. The siphonal capsule is well-developed, broad, and flat, with the apex of the siphon having hooked processes.

Tegmen: The tegmen has elongated, long lateral lobes with dense hairs; the median lobe is shorter than the lateral lobes or parameres, and the apex of the median lobe is pointed.

Micraspis yasumatsui (Fig. 9)

Sipho: The siphonal tube is strongly curved for most of its length. The siphonal capsule has an outer arm with a constriction, while the inner arm is short and rounded. The apex of the sipho is flattened.

Tegmen: The tegmen has elongated, long lateral lobes with dense hairs.

The siphonal tubes of above mentioned five commonly recorded coccinellids is presented in Fig-10 for better understanding of its structural differentiation between species to species.

In agriculture, accurate identification of insect pests, their bioagents, pollinators and other beneficial insects is of fundamental importance for effective, sustainable pest management and conservation of beneficial fauna (Poorani, 2022). Many researchers have conducted surveys and reported findings of coccinellid beetles from various regions worldwide (Khan *et al.*, 2009; Majumder *et al.*, 2013; Chowdhury *et al.*, 2015; Lami *et al.*, 2016; Halim *et al.*, 2017; Gurung *et al.*, 2019; Sharma and Joshi, 2020). The predaceous coccinellid beetles (11 species) reported in this study were collected from aphid hosts in different crop ecosystems at Pundibari. True aphidophagous coccinellids were first described by Agarwala and Ghosh (1988) as 36 species in the Indian subcontinent, and later by Poorani (2002) as 5,200 species worldwide. Coccinellid beetles in the Indian subcontinent are classified into 79 genera and 400 species. In 2020, Pervez *et al.* reported 18 predaceous coccinellid beetle species belonging to 15 genera and 3 subfamilies: Chilocorinae, Coccinellinae, and Scymninae. Polymorphism in insect groups has been a challenge for entomologists in achieving accurate species level identification. In coccinellid, this issue has garnered only limited attention, focusing on a few species (Honek, 1996). For instance, *C. sexmaculata* is the most ubiquitous coccinellid in the Indian region, yet its polymorphic forms are often misunderstood due to superficial similarities with other common but unrelated species, such as *M. discolor*, *Propylea dissecta* and *Chilocorus*

Fig. 4 Male genitalia (siphonal tube) of two morphs of *C. sexmaculata*

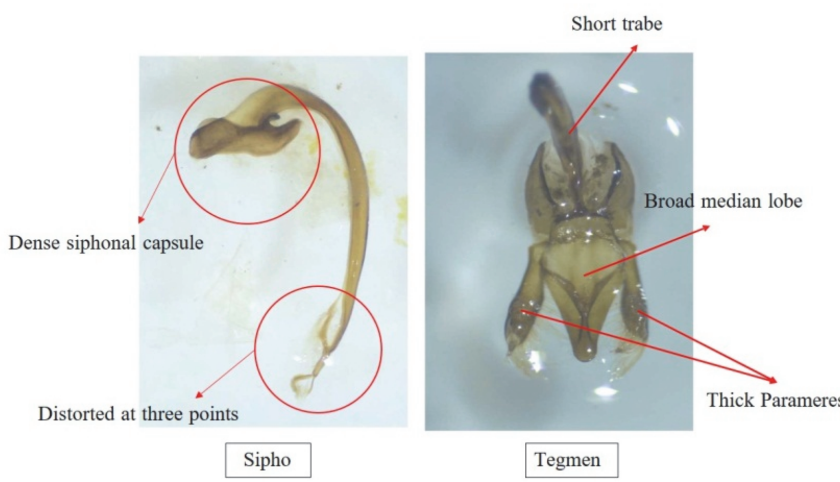

Fig. 5 Male genitalia structure of *Coccinella septempunctata*

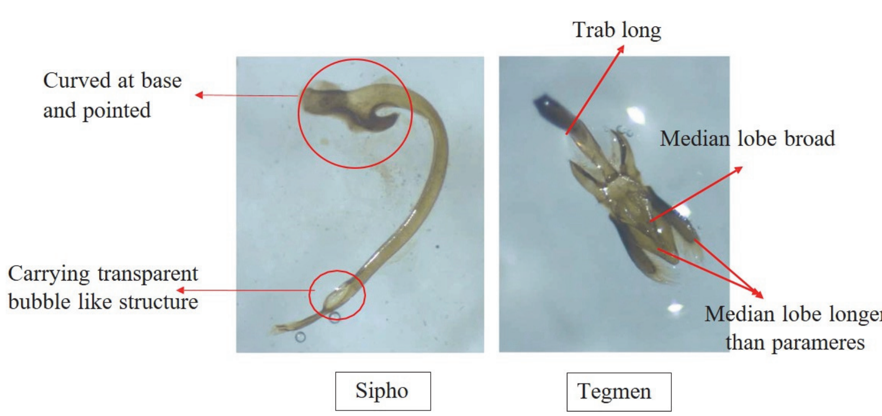

Fig. 6 Male genitalia structure of *Coccinella transversalis*

Fig. 7 Male genitalia structure of *Cheilomenes sexmaculata*

Fig. 8 Male genitalia structure of *Micraspis discolor*

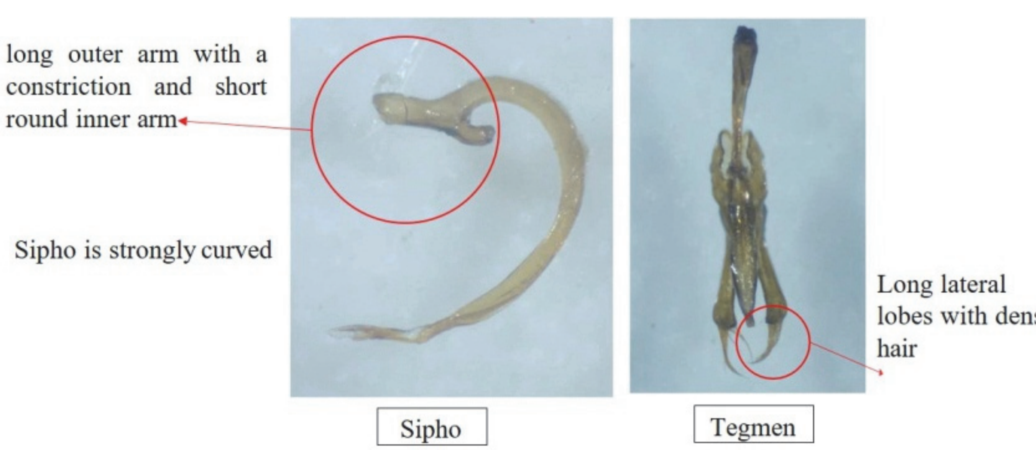

Fig. 9 Male genitalia structure of *Micraspis yasumatsui*

Fig. 10 Male genitalia Sipho of common coccinellids

nigrita (Poorani, 2023). In our present investigation, four different morphs of *C. sexmaculata* were recorded with the melanic forms (morph 3 and 4) being particularly confusing due to their resemblance to the external morphology of *C. nigrita*. This confusion could only be resolved by examining the male genitalia. Chazeau (1980) investigated the genetic causes of variation in the elytral colouration pattern in *Coelophora quadrivittata* in various related studies. In Chegeni region (Lorestan province, Iran), Biranvand and Shakarami (2015) identified 18 distinct morphs of *Hippodamia vareigata* Goeze based on variations in the elytra and pronotum patterns. Kawakami *et al.* (2013) documented 20 morphs of *Cheilomenes sexmaculata* from Indonesia to Japan, attributing elytral coloration to morph selection by climate. Similarly, Singh *et al*. (2016) reported six morphs of *Cheilomenes sexmaculata* in Haryana and Thamseer *et al*. (2022) found five morphs of *Coccinella septempunctata*. The genetic make-up of these forms, the varying environments to which they have been exposed, or a combination of both factors may be the cause of their variety.

In the terai sub-Himalayan region of West Bengal, eastern India, the common predaceous coccinellids encountered include *C. transversalis*, *M. discolor*, *C. sexmaculata*, *C. septempunctata*, *Brumoides suturalis*, *Propylea dissecta*, *M. yasumatsui* etc. (Gurung *et al*. 2018). The study of the male genitalia structures of these common species, revealed to give accurate identification of the coccinellid taxa at the species level.

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