



## Functional Morphology and Characters of five abundant species of Ladybirds (Coleoptera: Coccinellidae)

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**Abstract:** Functional morphology of five coccinellid species, viz. *Coccinella septempunctata*, *Coccinella transversalis*, *Menochilus sexmaculatus*, *Propylea dissecta*, and *Hippodamia variegata*, of the Coccinellini tribe (Coleoptera: Coccinellidae). These ladybirds prevalent arise in the agricultural fields, hence could be efficiently spotted by their characteristic elytral pattern and spots. However, we have dissected male and female genitalia and found significant differences at the species level. In males, paramere and median lobe while stylus and genital plate in females are exploitable to differentiate species exhibited by line-drawings.

**Keywords:** Coccinellinae, Coccinellini, genitalia, ladybirds, morphology, morphometrics.

### Introduction

Despite being gain popularity, biological control holds an important account for harvesting, there seems to be little research conducted about farmers perceive natural enemies on-farm (Martínez et al. 2020). Furthermore, the indirect benefits of biocontrol for instance yield were not particularly well understood by the farmers hence proper identification is a crucial step (Tüzün et al. 2010). It is believed that taxonomy is a complicated process and we agree to some extent that the morphological variation, except within categories, the task of taxonomic identification varies significantly. The task of identifying higher taxonomic groups seems to be challenging than the separation of species, even though certain species are quite similar (Valan et al. 2019). This could perhaps be done by visualizations of morpho-space, which are optimized to identify the species (Valan et al. 2019). Hence,

interactive visuals and identification of species contribute to a holistic approach for the general public. Zoogeographically, the north region is probably the richest in terms of faunal diversity and predominantly oriental, with strong Palaearctic regions (Ramani et al. 2019). Uttarakhand is home to a diverse range of biodiversity. In addition, principal revenue comes from agriculture as following conventional fostering management, but the inadequacy of practical implementing integrated pest management has still a long way to go.

Bulk literature regarding predaceous ladybirds has been surveyed by taxonomists. Undoubtedly, morphology-based taxonomy and systematics are undergoing many transformational changes. Numerous taxonomists had done sophisticated research on ladybirds emphasized by the release of the annotated checklists, which gather the scientific names, synonyms, distributions and



also provide accounts of morphological characters with illustrations of all living ladybirds species (Poorani 2002; Ślipiński 2013; Jafari et al. 2015). Predaceous Coccinellidae comprises of ladybirds, which prey upon numerous phytophagous pests, particularly aphids, diaspids and coccids (Pervez et al., 2020a). They even attack conspecifics (Pervez and Chandra, 2018) and heterospecific (Pervez and Kumar, 2017) ladybirds when natural prey is scarce. They maximise their impact on their prey, which is an important element in modern conservation biological control of indigenous natural enemies in contrast to the classical approach of releasing alien species (Hodek et al. 2012) and commercial worth by implementation of integrated pest management programmes (Hodek and Honěk 2009, Pervez et al. 2020b) since their life span is short and easy to culture by *ex-situ* methods (Hesler et al. 2012). Dixon (2000) emphasized the intricate prey-predation interactions of Coccinellidae and discussed the possible effects of different environmental factors on aphid biocontrol. The pronotum colour, pattern, and spots on elytra are used for distinguishing species but due to polymorphism, this should be not enough as they create turbulence for taxonomists. In different coccinellids species, abdominal shape, size and colour are strong morphological characters. Therefore, Rahaman et al. (2015) found that the abdominal segment characteristics can be employed for ladybird identification, and morphological variation might seem associated with industrial

contamination, behavioural diversity, and climate factors. The colour and its pattern vary greatly within the species with local adaptations resulting in increased, frequency of melanic morphs than the typical ones (Michie et al. 2010). For people who study and work on biodiversity aspects, the availability of species identification guides is the most essential requirement. However, guides to identify species are limited and available for only a few taxonomic groups (SCBD 2010). Consequently, the other features of organisms (such as distribution, ecology, biology) remain poorly known (SCBD 2010).

Therefore, our study focused on to provide detailed information on the functional morphology, to create initiatives to classify the most discriminating characters to develop farmers' knowledge concerning ladybirds and to eliminate confusion related to scientific names by providing synonyms list.

## Materials and methods

### Taxon selection

It originates from the initial intention to provide feasible identification guides as the main objective, we have restricted the taxonomic coverage to the tribe Coccinellini. Coccinellini comprises substantial predators and dominant biocontrol agents (Mandour et al. 2011). Mostly feeds on aphids, and scale insects lay their eggs singly or in patches. Adult males and females look more or less the same. They are about the same size, or female is a bit larger and lives longer than the male adult. Jouveau (2018) delineated it was



decided to focus on the Coccinellini tribe amidst their relatively low species diversity, as well as their large size and high colour diversity, that further initiated them easy to observe and collect by non-specialists.

### **Interactive field identification key to genera of the tribe Coccinellini**

#### **Adult**

1. White spots on black pronotum, elytra spots and pattern covered in short hairs – *Hippodamia* Goeze
2. Asymmetrical scape of antennae; whitish-yellow pronotum with pattern, black spot, dark sutural elytra band - *Cheilomenes* Fabricius
3. Without distinct ridge posterior margin of pronotum; drop like scutellar spot, postcoxal line that fuses with the same sternum's back margin via longitudinal line - *Coccinella* Linnaeus
4. White marks on black pronotum, white marks on scutellum both sides, body colour ranges from bright to dark orange and curvy pattern - *Coccinella* Fabricius
5. Black-brownish pronotum, decorated with spots and bands on elytra, mid-dorsal line of the junction - *Propylea* Mulsant

#### **Larva**

More or less alligator-like general appearances, elongate body (campodeiform), body colour grey to dark chocolate brown or black, with red, orange, yellow, or white spots, covered with a speck of whitish dust.

### **Collection and Preservation**

Adult ladybirds were gathered from the agricultural fields located in the Nainital (Uttarakhand), including Ramnagar. They were collected in the golden hour, between November 2020 to December 2020. These specimens were collected by random selection using sweep nets and hand collection method (Ammann et al. 2020). They were placed in glass vials with cotton plugs soaked with ethyl acetate for killing purposes, compiled by hardened, stretched, and labelled with consequential information about host plants, habitat, and collecting date. The specimens were kept in collection boxes and naphthalene balls were added to protect them from infections.

#### **Identification**

Standard identification keys, colour and shape based morphological attributes are defined primarily which follows Roy et al. (2013) nomenclature. Characters restricted only visible to the naked eye or with magnifying glass are comprehended.

#### **Genitalia extraction**

To separate and isolate the genitalia, Chelliah (1965) modified methodology, and the protocol was followed.

For 1 to 2 minutes, the specimens were placed in warm water. The abdomen was removed by fine needles and the extra tissues from the body were dissolved for about half an hour by boiling in a solution of 10% potassium hydroxide. The abdomen was washed in warm water and subsequently transferred to ethanoic acid for 5 minutes and followed by another 5 minutes washed in 70% ethanol (ethyl alcohol)



and again, another 5 minutes in absolute alcohol for complete dehydration. Finally, the abdomen was put in clove oil. The processed abdomen was put in a cavity slide, a drop of DMHF (Diethyl hydantoin formaldehyde, resin) on a slide, and placed with a specimen. It was dissected with fine needles under a stereoscope and the genitalia was detached (Fig-1). The length and width of the body parts, antenna, elytra and genitalia were

measured using a micrometre attached to a computer (DELL) for imaging. The data, body length and width, antennal length, and elytral length and width of the adult female of each ladybird species with ten replicates (n=10) were subjected to perform the Kolmogorov-Smirnoff test for Bartlett's test for homogeneity of variances using SAS (2002). Turkey's test and ANOVA were used for the comparison of means (Table 1).

**Table 1: Morphometric measurements (in mm) of adult females of five ladybird species of Uttarakhand.**

Ladybird Species	Body Length	Body Width	Antenna length	Elytra length	Elytra width
<i>C. septempunctata</i>	6.90 ± 0.73a	5.60 ± 0.96a	1.17 ± 0.07a	5.80 ± 0.25a	5.35 ± 0.24a
<i>C. transversalis</i>	6.50 ± 0.52a	4.70 ± 0.34b	0.74 ± 0.03d	5.25 ± 0.26ab	5.14 ± 0.35a
<i>M. sexmaculatus</i>	4.80 ± 0.63b	4.20 ± 0.53bc	0.63 ± 0.06e	4.15 ± 0.52b	3.35 ± 0.09b
<i>P. dissecta</i>	4.65 ± 0.62b	3.80 ± 0.34c	1.06 ± 0.03b	3.80 ± 0.48bc	3.14 ± 0.12b
<i>H. variegata</i>	4.45 ± 0.72b	3.65 ± 0.57c	0.92 ± 0.02c	3.45 ± 0.72c	2.87 ± 0.16b
F-value	30.78	17.28	181.95	15.25	7.38

Data are Mean ± S.D.; n = 10.

Tukey's Range = 4.02; d.f. = 4, 49

Means followed by different letters denote that the data is significant

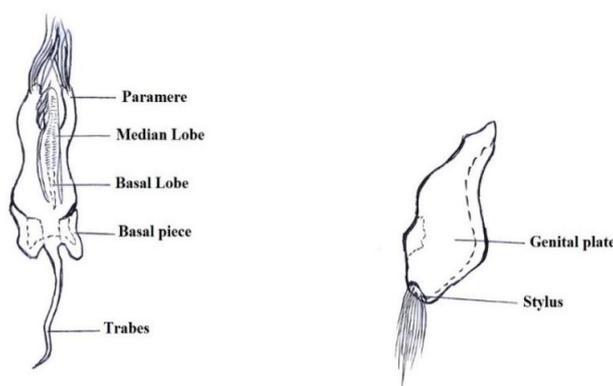


Figure -1 : General structure of adult (a) male and (b) female genitalia of predaceous ladybirds.

**Results and Discussion**

A total of 63 specimens were collected of five aphidophagous species.

***Coccinella septempunctata* Linnaeus**

*Coccinella 7-punctata* Linnaeus, 1758:365.

***Coccinella***

*septempunctata*:

Korschefskey, 1932:486.

*Coccinella divaricata* Olivier, 1808:1001.

*Coccinella confusa* Wiedemann, 1823:72.

*Coccinella confuse* Mulsant, 1850:112



*Coccinella bruckii* Mulsant, 1866:90; Crotch, 1874:46.

*Coccinella septempunctata* brucki: Korschefsky, 1932:491.

*Coccinella divaricata* Mader, 1936:375.

#### **Prototype examined**

Ramnagar, 2020.xi.04 ♀♂. On mustard, coriander, rose.

#### **Diagnostic characters of adult**

**Body:** Bilateral symmetry, a bit elongated oval like structure.

**Head:** Blackeyes and head. Two dark brown club-shaped antennae, 11 segmented with scape, pedicle and large flagellum. Black or dark brown colour mouth append 6 jaws, 4 maxilla, 4 lower lip, dark labrum; bifid mandible apically with a whitish spot at the lateral margin, near the base.

**Thorax:** Seven spots, three elytra spots extended to each side and one sutural spot just behind the middle of the thorax. The elytra colour is reddish-orange to bright red and has two yellowish patches on both sides of the scutellum black spot, the lateral side with a visible ridge and the posterior with a fine ridge. There are no hairs on the black elytra. Middle and hind legs are charcoal black, each has two spurs; unguis along with the basal subquadrate tooth.

**Abdomen:** Each of the sexes has six abdominal sterna covered posterior margin, convex in the female, blunt in the male, the six abdominal tergites curved in the female over posterior margin, strongly convex in the male.

**Genitalia:** Male sternum apodeme 9 rod-like, and chitinized; tegmen with asymmetrical short trabes, lateral lobes are somewhat smaller than basal lobe. Parameres were relatively shorter than the median lobe. Siphonal capsule bulged out and thick, outer arm comparatively thick than the inner arm. Siphonal tube long, with sac-like structure at the distal end. Female nearly semicircular spermatheca and broad genital plates.

#### ***Coccinella transversalis* Fabricius**

*Coccinella transversalis* Fabricius, 1781:97; Iablokoff-Khnzorian, 1979:68.

*Coccinella repanda* Thunberg, 1781:18.

*Coccinella madhuri* Sathe & Bhosale (p.42)

*Coccinella bhuimungi* Sathe & Bhosale (p.47)

*Coccinella yaminii* Sathe & Bhosale (p.52)

#### **Prototype examined**

Ramnagar, 2020.xi.24 ♂♀. On chilli, mustard, spinach.

#### **Diagnostic characters of adult**

**Body:** Elongated body.

**Head:** Black head. Pronotum with orange spots on antero-lateral side. Mouthparts are brown, 6 jaws, 4 maxilla, 4 lower lip.

**Thorax:** Yellowish-brown spots in every elytra. For the first 2 patches, the size arranges in ascending order and finishes with the round patch. The large black longitudinal stripe



along the inner joint of the elytra. The legs are black.

**Abdomen:** Abdomen small, pubescent, terga covered completely by the elytra, ventrally 6 abdominal sterna visible, 1<sup>st</sup> visible abdominal sternum has curved coxal line, depression is present on the last abdominal sternum.

**Genitalia:** Short thick trapezoid and its distal end are expanded. Quadrangle basal piece. Thick and long phallobase in males. The median lobe is comparatively longer than parameres, which are slightly compressed at the base and possess short hairs dorso-apically. Parameres are short and thick regardless of an ovoid tip with an extended bristle. Flat straight like genital plate in female.

***Menochilus sexmaculatus* (Fabricius)**

*Coccinella sexmaculata* Fabricius, 1781:96.

*Cheilomenes quadriplagiata* Dejean, 1837.

*Cheilomenes sexmaculata*, Mulsant, 1850:144.

*Menochilus sexmaculatus*, Timberlake, 1943.

*Menochilus quadriplagiatus*, Timberlake, 1943.

*Cheilomenes sexmaculata*, 1932:120.

*Cheilomenes hiugaensis* Takizawa

*Menochilus tuljapurensis* Sathe & Bhosale (p.75)

*Menochilus shivajensis* Sathe & Bhosale (p.79)

*Menochilus kharipi* Sathe & Bhosale (p.84)

**Prototype examined**

Ramnagar, 2020.xi.20 ♀♂. On coriander, rose.

**Diagnostic characters of adult**

**Body:** Vary from oval to round in shape. Honey colour with a sheer touch.

**Thorax:** Brown holds maximum portion in the head followed by yellow. The white straight horizontal line intersects with a small vertical line on the pronotum. Black-brown pigmentation of scutellum. Two zig-zag black lines without interaction. Elytra possess six black patches of geometrical configuration, including one on the posterior end. Only a black sutural stripe in elytra with red/yellowish/orange/pink in colour. The elytral markings coalesce and form broader bands. Elytra and pronotum are partially black leaving only the margins. Clavate antennae of male and female bearing three parts: scape, pedicel and 9-segmented flagellum. Ochraceous legs.

**Abdomen:** Both sexes, six visible abdominal sternites have, moderately convex in the female, while blunt in the male.

**Genitalia:** Apodeme of male sternum 9 not chitinized, rod-like; tegmen slightly chitinized, with long asymmetrical trapeses; basal lobe elongated. Parameres are significantly longer relative to the median. Coxites elongated, slightly chitinized. The siphonal tube shape is remindful of the pinna. Female spermatheca worm-like. Swan like the genital plate and wide stylus.

***Propylea dissecta* (Mulsant)**

*Propylea japonica* ab. *dissecta* Korschefsky, 1932

*Lemnia (Vola) dissecta* Mulsant, 1850

*Lemnia mystacea* Mulsant, 1853

*Harmonia feliciar* Mulsant, 1866

*Propylea fallax* Iablokov-Khinzoryan, 1977



### Prototype examined

Ramnagar, 2020. xii.14♂♀. On fenugreek, spinach.

### Diagnostic characters of adult

**Body:**Egg-shaped, and black on the ventral side. Black-brownish head.

**Thorax:**It has scutellum and pronotum which are blackish pigmentation. Clavate type antennae. Each elytron has two strips cause of the species multiformity.

**Abdomen:**Small pubescent abdomen. Terga are covered completely by the elytra and six abdominal sterna ventrally visible. Posterior margin of abdominal sterna smooth, 1<sup>st</sup> visible abdominal sternum has curved coxal line.

**Genitalia:** In males, phallobase is moderately long, curved and slender trab. Normal parameres. Uniformly thick median lobe. Siphonal capsule arm not equal to parameres and convoluted in the form of hair-like structure. Curved shaped genital plates.

### *Hippodamia variegata*(Goeze)

*Coccinella variegata* Goeze, 1777:247.

*Adonia variegata*, Mulsant, 1846:39.

*Adonia variegata*: Koreschefsky, 1932:346-359.

*Adonia variegata*: Kapur, 1942: 346-359.

*Hippodamia variegata*: Belicek, 1976:338.

*Hippodamia* (Adonia) *variegata*: Iablokoff-Khnozorian, 1982:326.

### Prototype examined

Ramnagar, 2020.xii.21♂♀. On coriander, rose.

### Diagnostic characters of adult

**Body:**Round and amber coloured body. An eye-catching white pattern appears on the soldier's head, while white spots appear to be eyes.

**Thorax:** Highly polymorphic with varying elytral spots and size related to altitude. 3 dots make the triangle reciprocal as well as 1 prominent dot on the median line of the back (total of 13 spots). Without hair. White mark with yellow tint at the end. 11 segmented antennae and 3 segmented labial palpi. Black to dark brown forelegs coxae with two whitish spots in females, and brownish yellow in males. Legs similar in shape with 3 segmented tarsi, black or dark brown.

**Abdomen:**Ventrally 5 abdominal sterna visible, black or brown. Convex abdomen in female while truncate in the male.

**Genitalia:** Apodeme of male sternum 9 absent. Symmetrical parameres and long phallobase trab. Female coxities club-handle like. Infundibulum absent. V-shaped stylus and flask like a genital plate.

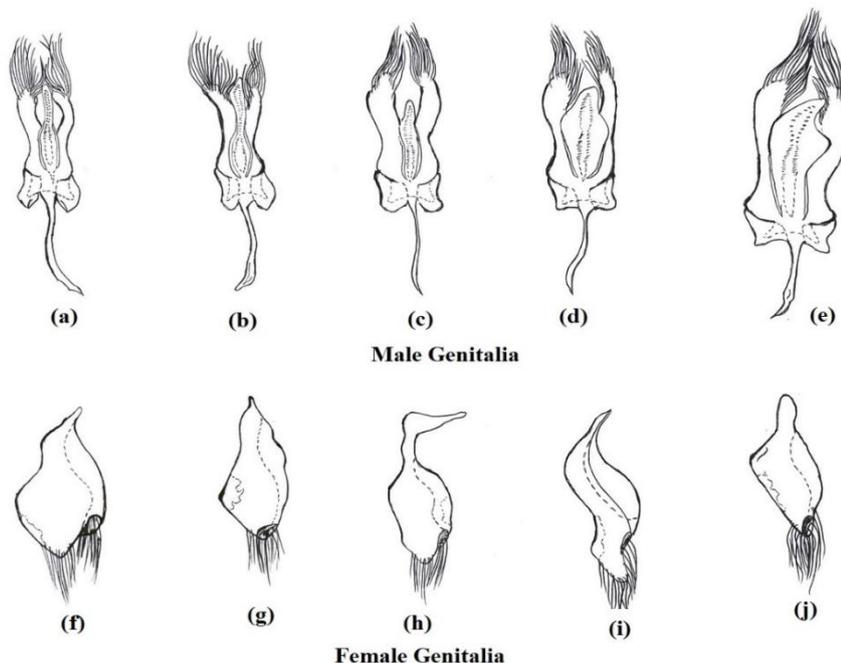


Figure-2: Schematic drawing showing the male (a-e) and female (f-j) genitalia of *C. septempunctata* (a, f), *C. transversalis* (b, g), *M. sexmaculatus* (c, h), *P. dissecta* (d, i) and *H. variegata* (e, j).

## Conclusion

Coccinellini tribe fauna of Uttarakhand has shown great intraspecific diversity in terms of body size, the colour of elytra, the number of spots on elytra, pronotum colour and establish a standard comparison. *C. septempunctata*, *P. dissecta*, and *H. variegata* show a high degree of polymorphism. Only *M. sexmaculatus* and *H. variegata* have rounded body while others are elongated. *C. septempunctata*, *C. transversalis*, *H. variegata* are black-headed while *M. sexmaculatus* and *P. dissecta* yellowish brown/black-headed. Elytra colour, pattern, and lines are critical for field identification. As for genitals, only *C. transversalis* has a longer median lobe than paramere. On top of, male genitalia and siphonal tube resembling carpel and stamen in appearance. Henceforth the male and female genitalia have various characters that are important for differentiating species at the

level of interspecific or intraspecific. Occasionally farmers misinterpret larvae of ladybirds for pests and spritz chemical pesticides that have lethal effects. It's due to a deficit of information and increased problems. The only way to combat this problem is a constant educational effort to inform people about ladybirds and biological control methods. However, due to limited systematic knowledge of ladybirds, there is a gap of knowledge between theory and practical application. This paper prevails only a small portion of morphology and a lot more to be found.

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