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Morphological Study of Male Genitalia in Ladybird Beetles from Nashik District, Maharashtra

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Abstract: The family Coccinellidae, belonging to the order Coleoptera, is ecologically and economically significant due to its dual roles as predators and phytophages. This investigation focuses on the diversity and distribution of Coccinellid beetles in the Nashik district of Maharashtra. A total of 160 beetles were documented, representing six predominant predatory species. These species fall under subfamilies including Coccinellinae, Chilocorinae, Coccidulinae, and Epilachninae. *Cheilomenes sexmaculata* (Fabricius) emerged as the most prevalent species, with 185 individuals observed across multiple host plants throughout the study period. The next most frequently encountered species was *Hippodamia convergens*, with 180 specimens recorded. *Hippodamia variegata* was found the dominant species of all the predatory coccinellids with 210 specimens collected from different crops and plants. This species was found throughout the survey period. The 2nd most common species was *Illies cincta* with 130 species specimens collected on available crops and vegetables. This species also was found throughout the study area.The members of *Anegleis cardoni* were collected from Cucumber, weeds, alfalfa and grasses. *Cryptolaemus montrozieri* specimens were found quite rare in district Nashik. All the observed species have been described for the first time from Nashik district. The presence of *Cryptolaemus montrozieri* was good sign for agriculture because it is fungivorous. Six species exhibited broad temporal and spatial distribution.

Keywords: Coleoptera, Coccinellidae, Biocontrol, Nashik, Genital Morphology

I. Introduction:

The extensive use of synthetic chemical pesticides in modern agriculture has contributed to environmental degradation and poses risks to both target and non-target organisms. As a sustainable alternative, biological control methods, particularly those involving predatory insects such as ladybird beetles (family Coccinellidae), have gained prominence. These beetles are known for their effectiveness in controlling aphids, whiteflies, mites, and other phytophagous pests during both larval and adult stages.

Coccinellid beetles are generally small, oval-shaped insects, often adorned with distinctive coloration and spotting patterns. Globally, approximately 5,200 species have been identified. Their diagnostic traits, especially the male genitalia, are highly species-specific and serve as a reliable tool for taxonomic differentiation in the absence of distinctive external morphology. The morphology of male genital structures—typically complex and heavily sclerotized—has long been utilized in coleopteran taxonomy and phylogenetic studies, tracing back to early foundational work by Sharp and Muir (1912) and more recent advances by Richmond et al. (2016).

The Coccinellidae is the family of small lady beetles, ranging from 0.8 to 18 mm (0.315 to 0.708 inches), they are commonly yellow, orange or scarlet with small black spots on their wing covers, with black legs, heads antennae, such as Vibidia decinguttata. A twelve-spotted ladybeetle species, have whitish spots on a brown background. Coccinellids are found worldwide, with over 5,000 species, described. Structure of male genitalia is species-specific and is the most important diagnostic character, in the absence of other reliable external diagnostic characters. Insects genitalia are complex and provide basis for species discrimination in most families and helps in family identification which are attached to the distal segments of the abdomen (Dobzhanskiy, 1926; Timberlake, 1943; Kapur 1958; Chapin, 1965; Shah and Khan, 2014). The uniqueness of male genitalia of a species lead to the use of the morphological study of genitalia as one of the most important keys in taxonomic identification of taxa below family level (Hoskins and Adrian, 2010; Chowdary *et al*., 2015). Male genitalia of insects tend to be heavily sclerotized, rigid structures that are seemingly more diverse than female reproductive morphology, where species-specific male traits are more apparent to human investigators and are usually the primary focus of genital evolution studies (Richmond et al., 2016). Structure of male genital tube in the order, Coleoptera was first time used as an important taxonomic tool, not only for the discrimination of different species of beetles, but also their potential use in tracing out the phylogeny of various groups (Sharp and Muir, 1912).

Maharashtra, and in particular the Nashik district, lies within the biodiverse region of the Western Ghats, a globally recognized biodiversity hotspot. Despite this richness, detailed taxonomic studies on Coccinellidae, particularly focusing on genital morphology, are scarce for this region. Given the importance of accurate species identification for biological control implementation, this study aims to describe and document the morphological characteristics of male genitalia in eight species of ladybird beetles found in the Nashik region.

The coccinellid diversity of North Maharashtra is rich but study on taxonomy is far lacking. The knowledge of their habitat and diversity will benefit in identifying the predators and eventually use as biocontrol agents.

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The Nashik region is the well-known for the cultivation of variety of crops kike grapes, bananas, onion, sugarcane, pomegranate, cauliflower, cabbage, maize, cotton, wheat. Western region is mainly a tribal hilly area covered with the variety of forests. This variety of flora invites many insect pests of agricultural crops and vegetables, which causes heavy damage to them. The cultivated crops like maize, wheat, grapes, cotton and vagetables are infected by both sucking and chewing type of insects. The sucking type of insects like white flies, aphids, mealybugs, mites and chewing type of insects like grasshopper, termites.

II. Materials and Methods:

Study Area

The study was conducted in the Nashik district of Maharashtra, India, a region renowned for its ecological diversity. Geographically, Maharashtra forms a large triangular area bounded by the Arabian Sea. The selected study area included the Satana and Kalwan talukas within Nashik district. This region is part of the Western Ghats (also known as the Sahyadri Hills), which run parallel to the western coastline of India and average 1,200 meters in elevation (Gurule, 2013). Recognized as one of the 25 global biodiversity hotspots (Daniels, 2011), the Western Ghats are second only to the Himalayas in terms of ecological significance in the Indian subcontinent (Gaonkar, 1996).

Sample Collection

To assess the diversity and ecological characteristics of Coccinellidae, ladybird beetles were sampled from four different locations within Satana and Kalwan talukas. Surveys were conducted between January 2024 and February 2025 across agricultural fields and natural vegetation infested with common crop pests such as aphids, whiteflies, and mealybugs. All cultivated and wild vegetation in the study areas was examined. Adult beetles were collected at monthly intervals using hand-picking and sweep netting techniques during early morning (6:30–9:30 AM) and evening hours (4:30–6:30 PM). Data were systematically recorded on a structured field datasheet.

Specimen Preservation and Identification

Collected beetles were transported to the laboratory in breathable containers. For euthanasia, specimens were placed in insect-killing jars containing cotton balls soaked in ethyl acetate. After death, specimens were preserved in 70% ethanol for subsequent morphological studies. Identification was conducted using standard taxonomic keys and verified with reference to authenticated literature.

Specimen were brought into the laboratory and killed in insect killing jars by placing an ethyl acetate-soaked cotton ball in them and closing them until the insect died.

Dissection and Preparation of Male Genitalia

Dissection followed a modified version of the procedure described by Majerus and Kearns (1989). Initially, dried beetles were softened by immersing them in hot water for 1–2 minutes. The abdomen was carefully detached using fine entomological needles and boiled in a 10% potassium hydroxide (KOH) solution for 30 minutes to clear internal tissues. Following this, specimens were rinsed and soaked overnight in lactophenol to dissolve residual tissues and enhance transparency.

For examination, the processed abdomen was transferred to a cavity slide containing glycerin. Dissections were performed under a stereozoom binocular microscope. The extracted male genitalia were mounted on a cardboard strip using water-soluble adhesive, allowing detailed observation of structural characteristics. This preparation rendered the entire genitalia complex visible and suitable for comparative morphological study.

Observations and Results:

The main objective of the present research work was to explore the fauna of coccinellids and their distribution in Nashik district. Present studies on structure of male genitalia of 6 different species of ladybird beetles revealed that structure of the male genitalia is species-specific having parts viz., tegmen and sipho and was found to be the most important diagnostic character for identification of ladybird beetle species. However, for taxonomic identification, male genitalia varied structurally in terms of shape of the siphonal capsule, basal or median lobe being symmetrical or asymmetrical, shape of the parameres, length of the parameres in comparison to median lobe and shape of the median lobe which showed variation.

The primary objective of this study was to document the diversity and genital morphology of Coccinellid beetles inhabiting the Nashik district. Examination of male genital structures from four distinct ladybird beetle species revealed that these structures are highly species-specific. Diagnostic features included the tegmen and sipho, both of which proved crucial for reliable species identification.

Morphological distinctions in the male genitalia were evident in terms of the symmetry and shape of the siphonal capsule, configuration of the basal and median lobes, paramere dimensions, and overall structural characteristics of the median lobe. These variations formed the basis for interspecific differentiation.

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Species Descriptions

Cheilomenes sexmaculata (Fabricius)

Morphology: Commonly known as the six-spotted zigzag beetle, adult specimens ranged from 3.3–6.2 mm in length and 3.0–5.3 mm in width. The beetles exhibited a broadly oval, moderately convex body shape with a glossy dorsal surface. Elytra were typically dark brown, while the head was dark and the pronotum displayed a central black T-shaped mark extending into a posterior black band. Elytra contained six distinct black maculae arranged in a zigzag pattern. Larvae were dark grey to brown with pale yellow patches, and pupae showed a yellow base coloration with black markings.

Male Genitalia: The sipho was strongly curved basally and tapered to a fine apex. The siphonal capsule was asymmetrical, with the outer arm longer and the inner arm shorter. The tegmen was broad and extended distally, with an oblong basal piece. Parameres were long, thick, and cylindrical, slightly curved at the base, and bore long hairs subapically. The median lobe was shorter than the parameres and thick along most of its length, with a straight outer edge and a slightly convex inner edge that narrowed apically. The ninth abdominal segment's apophysis was broad and bifid at the posterior end.

Hippodamia convergens

Morphology: Adults measured between 4–7 mm and had an elongate, narrow, and weakly convex body. The head was yellow with a transverse black marking, while the pronotum was black with white lateral margins and sometimes fused eye-like spots at the center. The scutellum was black, and the elytra were orange or red, marked with thirteen black spots, including one postscutellar and six lateral spots on each elytron. The ventral surface was entirely black. Both larvae and adults are voracious predators.

Male Genitalia: The phallobase trabe was short and wide, with a distinctly concave apex. The basal piece was quadrate and roughly equal in length to the median lobe. The median lobe and parameres were approximately equal in size. The siphonal capsule formed a simple loop, appearing tubular and widest at the center. Viewed ventrally, it displayed two protruding arm-like extensions that tapered sharply toward the apex.

Illies cincta (Fabricius):

Morphology: It is yellow colored beetle. The adult beetle is 5.2 mm long and 4mm broad across the middle elytra. Form elongate oval, moderately convex. Head pale yellow to creamy yellow in coloured. Eyes separated by more than one eye width. Pronotum with a pair of median black spots on posterior margin; anterior and lateral margins transparent, lateral margins slightly upturned. Elytra bright lemon yellow coloured. Ventral side more or less uniformly yellowish. Larva lemon yellow, with four longitudinal rows of black spots on dorsal side. Pupa pale yellow with areas of grey and black markings.

Male Genitalia:

Tegmen with elongated lateral lobes which was densely hairy at the apex, median lobe longer than lateral lobes. Sipho was strongly curved basally and apically. Apex of the sipho was spatula like and inner processes of siphonal capsule short and round, outer processes pointed. Apophysis of ninth abdominal segment remained elongated and bifid caudally.

Anegleis cardoni (Weise):

Morphology: The adult beetle Anegleis cardoni is ranges from. 3.50-3.75 mm. length and 3.25- 3.50 mm. width. Form round, strongly convex. Ground colour pale creamy to on lemon yellow. Pronotum with a pair of triangular markings on posterior margin and a pair of small, transverse markings in the middle. Scutellum minute, triangular and black. Each elytron with a pair of thin black stripes, outer one anteriorly bent inwards and inner one posteriorly bent autwards and a small circular spot near apex; sutural line with a black stripe; outer margins of elytra very narrow black. Ventral side uniform pale yellow. Postcoxal line on abdominal ventritel.

Male Genitalia: Trabe of Phallobase was short and distally thick. Basal piece remained long and rectangular. Median lobe was distally expanded; tip deeply excavated. Siphonal capsule was large, outer arm long and inner arm was small hook like. Siphonal tube was bent, long, thick at the base, then straight and gradually narrowed towards the apex.

Cryptolaemus montrozieri (Mulsant):

Morphology: Elongate, oval, convex covered with golden- grey dense pubescence. Head and pronotum brown without any pronotal spot. 10 segmented antennae. Elytra is black with apical horizontal corner brown (Plate V B; Image a). Ventrally, metasternum is black. 1 ^ (st) pair of leg is brown and remaining two pairs are black. Postcoxal line is complete (Plate V B; Image d). Tarsi in females are trimerous having bifid tarsal claw without basal tooth and in males it is appendiculte i.e. without any groove.

Male Genitalia: Penis curved, with uniform width (Plate V B; Image c - d) Penis capsule having longer inner arm but thicker than outer arm. Outer arm is short and broad. At half of the penis length, the outer margin of penisn is wavy with sub rounded outgrowths. Tegmen with long trabe (Plate V B; Image e-f). Penis guide is distinctly short than parameres. Parameres with apical half broad, balloon like swollen. Inner paramere margin covered with long dense setae.

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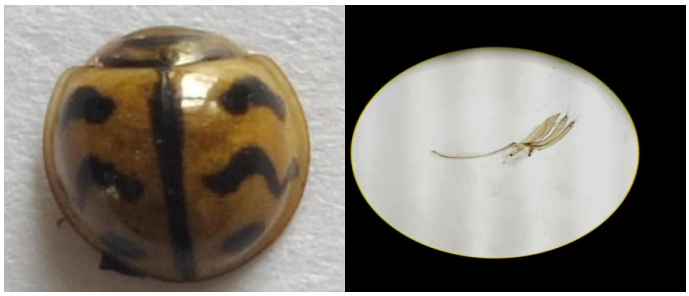
Hippodamia variegata (Goeze):

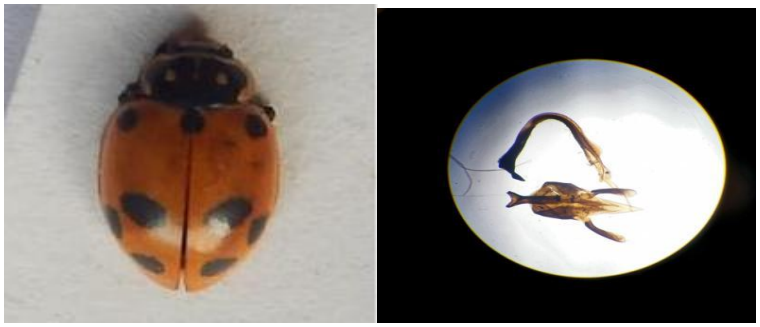
Morphology: The adult beetle measures 4-7 mm long. It is narrow, elongate, oval, and weakly convex. Head is yellow with a black transverse marking. Pronotum black with white anterolateral margins and a pair of median eyes, sometimes fused together. Scutellum is black in colour. Beetle colour is orange or red, with whitish/creamy yellow areas adjacent to scutellum and nine black spots are present on elytra, one postscutellar spot and four on each elytron. Ventral side more or less completely black. These beetles are predaceous as both larvae and adult. Their larvae are active, elongate and voracious.

Male Genitalia: Trabe of phallobase was short, broad, apex wide and deeply concave. Basal piece was quadrate, equal in length to median lobe and median lobe was equal in size to parameres. Siphonal capsule was simple, tube forming loop, wide in the middle, when seen from below two arm like structures were observed as protruded which abruptly tapered up to the apex.

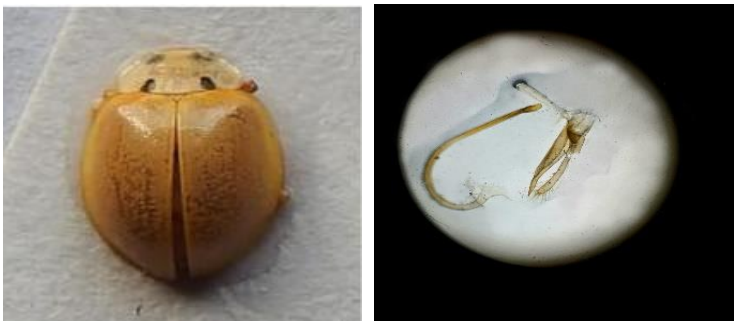
Photoplate 1



Cheilomens sexmaculata male genitalia



Hippodamia convergens male genitalia



Illies cincta male genitalia

Photoplate 2

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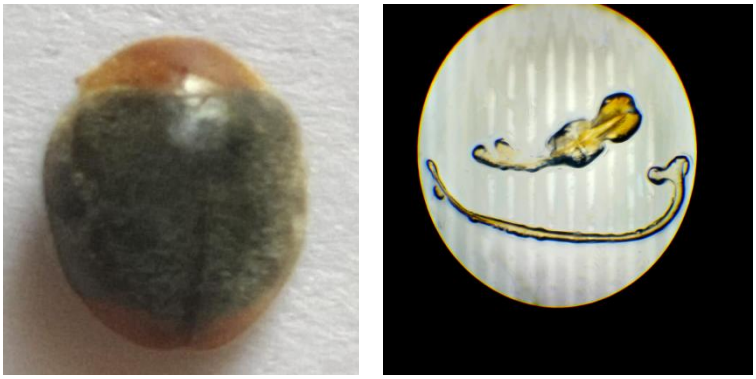
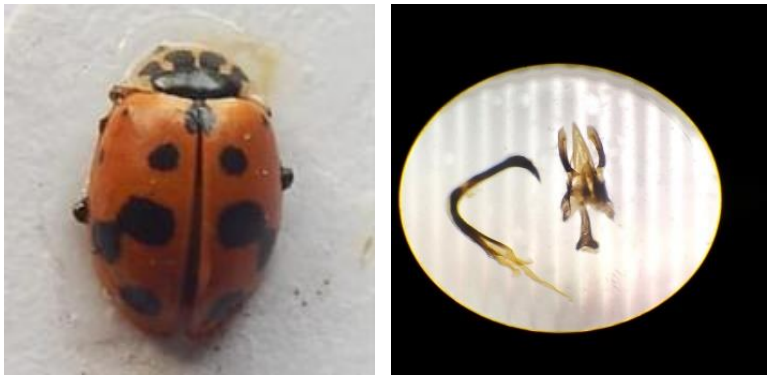
Anegleis cardoni male genitalia



Cryptolaemus montrozieri male genitalia



Hippodamia variegata male genitalia

III. Discussion:

The present study was conducted in district Nashik with main objective of exploring the Coccinellid species prevailing in the agro-ecosystem of the district. A total of 6 species of Coccinellid were found in vegetables, crop field and general vegetation of the study area. These species belong to 4 subfamilies and 5 genera. *Hippodamia variegata* is another common species of the study area, which is also highly polymorphic in nature. This species is abundant in wheat, Alfalfa and on grass.

The male genitalia of 6 different ladybird beetles were analysed belonging to the four subfamilies. Results obtained reflect the detailed dissections and observation of each part of the male genital structure that was being used in the taxonomy of the group. The characteristics of the male genitalia were used with great reliability for both identification and phylogenetic analyses for devising more consistent diagnostic characters for species and genera.

This study was undertaken to investigate the diversity of Coccinellid beetles within the agro-ecosystems of Nashik district, Maharashtra, with a particular emphasis on the structural variations of male genitalia. Six dominant species—*Cheilomenes sexmaculata*, *Hippodamia convergens, Illies cincta, Anegleis cardoni, Cryptolaemus montrozieri* and *Hippodamia variegata* — were documented, they widely distributed across crop fields and natural vegetation. These species belong to two genera and subfamilies, underscoring the diversity of the region.

Among the findings, *Cheilomenes sexmaculata* and *Hippodamia variegata* was observed as a polymorphic and highly adaptable species, commonly associated with crops such as wheat, alfalfa, and grass. The male genitalia of these species exhibited distinct morphological characteristics, highlighting their diagnostic significance. Dissections revealed structural differences in the siphonal capsule, parameres, and median lobe, which are essential for species-level identification.

Coleoptera remains the most diverse order among insects, accounting for approximately 277,125 species globally, or about 36% of all insect species (Banki et al., 2021). Within this order, the suborder Polyphaga includes the family Coccinellidae, which holds immense agricultural importance. Members of this family are primarily known for their role as natural enemies of pest insects, although a minority are phytophagous and may damage crops.

Among insects, the dominant and largest order is Coleoptera (2,77,125 species), contributing 36% of the class Insecta (Banki et al. 2021). This order distributed throughout the world consists of 114 families that belong to two suborders, Polyphaga and Adephaga, and in India it represents 4.86% of known species in the world. The family Coccinellidae of suborder Polyphaga is an economically important family of the Coleoptera. The family members of it are generally known as ladybugs, ladybirds or lady beetles in different parts of the world and are mostly predators that act as biocontrol agents for various crops. However, some species of Coccinellidae

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are pests of some of the crops. Two subfamilies and 6000 species under 360 genera of 30 tribes of Coccinellidae are described worldwide (Slipinski and Tomaszewska 2010). About 90% of the ladybird beetles are predators of crop pests and beneficial ones, and remaining 10% consists of mycophagous and phytophagous coccinellids, of which phytophagous serves as pests of crops. Predatory ladybird beetles' food preferences are mostly aphids, coccids, adelgids, mites, ants, aleyrodids, cicadellids, pentatomids, phylloxera, chrysomelid larvae and psyllids (Roy and Migeon 2010). According to the reviewed literature, it is clear that no one has thoroughly studied the diversity and specificity in male genitalia of the ladybird beetles from the study region, Nashik district, Maharashtra, India.

Globally, the Coccinellidae family is comprised of around 6,000 species classified under 360 genera and 30 tribes (Slipinski & Tomaszewska, 2010). Nearly 90% of these species are beneficial predators, feeding on soft-bodied pests like aphids, coccids, aleyrodids, and psyllids (Roy & Migeon, 2010). Despite this, research on the male genitalia of ladybird beetles in Maharashtra, and specifically in the Nashik district, remains limited.

The Nashik district is a part of the Northern Western Ghats—recognized as one of the world's prominent biodiversity hotspots (Daniels, 2011). However, comprehensive taxonomic surveys focusing on genital morphology in Coccinellidae have been largely absent from this area. Given that accurate species identification is critical for biodiversity assessment and implementation of biological control strategies, this study contributes essential baseline data. While expert morphological identification remains ideal, molecular methods can offer reliable alternatives in the absence of taxonomic expertise.

In the present research, systematic sampling was conducted at six different locations across the district using standard entomological methods. Specimens were preserved and dissected following accepted protocols, and identification was confirmed using recognized taxonomic literature. The results demonstrate that the genital morphology of male Coccinellids is not only species-specific but also holds potential value in phylogenetic and evolutionary studies.

IV. Conclusion:

The study region, Nashik district, is explored to investigate the diversity and variation of male genitalia of ladybird beetles. As this region is a part of one of the hotspots of the world i.e. the Western Ghats, and this hotspot has not explored thoroughly, it is essential to make an inventory of species diversity, which leads to the possibility of new species records.

During present investigation, a total of 6 species of ladybird beetles belonging to 5 genera of 9 tribes under 2 subfamilies, i.e. Microweisinae and Coccinellinae (according to revised classification by Seago et al. 2011), were recorded from the study region. The data collected during this study will serve as baseline data for the upcoming researchers who want to work in the fields of taxonomy, diversity and variation of male genitalia of ladybird beetles.

Most of the recorded species are predators of crop pests, acted as biological control agents. In all, 6 species of ladybird beetles were found on different crops of Nashik district indicating there is tremendous scope to implement biological control strategy against various crop pests of the Western Maharashtra including study region, Nashik district.

The record of 4 species of ladybird beetle from the study area within a period of one years indicates that Nashik district is rich in biodiversity and suitable for ladybird beetle survival. There is a need to create awareness among local farmers to protect and conserve these biocontrol agents. Exploration of the study area for a longer duration will add more species to the existing ladybird beetle fauna of Maharashtra as well as India, and may also add species new to science.

In the monsoon season, the ladybird beetle diversity is greater, but due to the drying of crops in summer, the crop pest population decreases, which leads to a decrease in ladybird beetle number and diversity in summer among ladybird beetles.

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