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Research Article

Diversity of coccinellid beetles (Coleoptera: Coccinellidae) in agricultural fields of northern Kerala, India

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ABSTRACT: Coccinellidae is a diverse and species-rich family that includes predaceous, phytophagous and mycophagous insects, which are significant to agriculture. This study aimed to estimate the diversity of coccinellids and document their associated prey in agricultural fields of northern Kerala, India and to generate DNA barcodes for the species collected during the study. Twenty-seven species under seven tribes were reported in this study, which includes three phytophagous, two mycophagous and 22 predaceous beetles. The information on prey species and/or host plants of the beetles are given furnished. Diversity indices of coccinellids across different agroecological zones and crops of northern Kerala were estimated. Mitochondrial cytochrome oxidase subunit I sequences of 18 species were generated and phylogenetic analysis was carried out. Coccinellini was recorded as the predominant tribe of Coccinellidae in northern Kerala. High hills were found to be the most diverse agroecological zone for Coccinellidae, whereas plantation crops and vegetables were the most diverse crop ecosystems.

KEYWORDS: Coccinellidae, species diversity, prey range, DNA barcoding, COI gene

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INTRODUCTION

Coccinellid beetles, commonly known as ladybirds or lady beetles, belong to the family Coccinellidae of the order Coleoptera, and play a significant and multifaceted role in agriculture, making them invaluable allies to farmers and ecosystems. Coccinellidae is the largest family within the superfamily Coccinelloidea (Robertson *et al*., 2015), comprising around 6,000 documented species globally under 360 genera in 30 tribes (Slipinski, 2007). Based on the morphological and molecular evidence, traditional six or seven subfamilies (Sasaji, 1971; Kovar, 1996) of Coccinellidae were categorised into two subfamilies, Microweiseinae Leng and Coccinellinae Latreille (Slipinski, 2007; Seago *et al*., 2011), Recently, Che *et al*. (2021) recognised three subfamilies viz., Coccinellinae, Monocoryninae Miyatake, and Microweiseinae under Coccinellidae.

Coccinellids are ecologically and morphologically diverse beetles and exhibit a wide range of food habits. The majority of the coccinellids are predaceous on various agricultural pests, while a few are phytophagous, and some are mycophagous. Both grubs and adults of predatory coccinellids feed on hemipteran pests viz., aphids, psyllids, whiteflies, leafhoppers, planthoppers, scales, and mealybugs, and early instar larvae of various insects, and also mites (Poorani & Krishnakumar, 2019). Coccinellidae is one of the most important groups of predators specific to many of these sucking pests and has the potential to control the densities of these insects in natural and agricultural ecosystems (Hodek & Evans, 2012). Phytophagous coccinellids, mainly epilachna beetles, are also economically significant as these cause considerable damage to a variety of agriculturally important crops. Mycophagous coccinellids of the genera *Illeis*, *Psyllobora*, *Halyzia* and *Tytthaspis* are known to feed on powdery and downy mildews (Poorani & Krishnakumar, 2019).

Knowledge on the diversity and relative abundance of coccinellid fauna in different agricultural ecosystems may provide insight into environment-friendly pest management strategies. The phenotypic plasticity and cryptic nature of coccinellids, often challenge their identification based on morphological characteristics. DNA barcoding, introduced by

Hebert *et al*. (2003) allows quick and accurate identification of species in insects including beetles (Pentinsaari *et al*., 2014; Halim *et al.,* 2017).

Objectives of this study include documentation of species composition and estimation of diversity indices of coccinellid beetles in different cropping systems and agro-ecological zones of northern Kerala, and to generate DNA barcodes of coccinellids collected during the study, which can be used as a reference in further biodiversity studies.

MATERIALS AND METHODS

Collection, preservation and identification of coccinellid beetles

Surveys were conducted during 2021-22 across four different agro-ecological zones, *viz*., coastal plain, midland laterites, foothills, and high hills, covering Kasaragod, Kannur, Kozhikode, and Wayanad districts of northern Kerala, India. Different crop fields, *viz*., rice, vegetables, fruit crops and plantation crops were covered during the survey, and beetles were collected by hand and net sweeping. The grubs and pupae were also collected during the survey and were reared to adults. To ensure comprehensive data collection, three locations within each crop field in a particular zone were surveyed. Samples were collected during two periods *viz*., December 2021 to May 2022 and June 2022 to November 2022.

In the rice field, beetles were collected by 10 net sweeps in each plot of 100 m × 25 m mostly during panicle initiation and flowering stages of rice. In addition, beetles were also collected from field bund (Shanker *et al*., 2018). In vegetable crops *viz*., cowpea, okra, brinjal, cabbage, tomato, and chilli, 10 plants within a plot of size 200m^2 were randomly selected, and beetles were collected by hand (Rajan *et al*., 2019). In case of cucurbits, a quadrant sampling method was followed for the collection of coccinellid beetles. Ten quadrants measuring 1m^2 were surveyed in each plot of size 200m^2 and the beetles associated with the cucurbit plants in each quadrant were collected by hand. In the case of fruit crops and plantation crops, ten trees from an orchard or plantation were randomly selected and beetles were collected (Kinawy, 1991; Rasheed & Buhroo, 2018). The collected beetles were kept in polybags and labelled with details of the host plant and location. The associated prey insects were also collected from each locality.

In laboratory, the beetles were grouped based on external morphology such as size, shape and elytra pattern, on observation under a stereo binocular microscope (Leica M80). The number of specimens in each group was recorded. The collected beetles were preserved following the standard procedure. Beetles were either dry preserved by mounting or wet preserved in 70% ethanol, for morphological identification. For molecular studies, the beetles were wet preserved in 99% ethanol at -80°C. The prey insects were wet and preserved in 70% ethanol.

Photographs of the habitus were taken using the Leica MC 170 HD camera attached to the microscope Leica M80 and software LAS. Composite images were generated and stacked using Combine ZM software. The coccinellid beetles were identified by Dr. J. Poorani, Principal Scientist, ICAR – National Research Centre for Banana, Tiruchirapalli and Dr. Vidya C. V., Kerala Agricultural University. The associated prey insects were identified by Dr. Sunil Joshi, Principal Scientist and Head of Division, Division of Germplasm Collection and Characterization, NBAIR, Bengaluru.

Locations surveyed during the study were represented as maps and were prepared using QGIS 3.30.3 software (Figure 1).

Estimation of diversity indices

The data on the number of beetles collected in each species from different agroecological zones/ crops in two periods were compiled and statistical analyses for diversity indices were done using PAST version 4.03 (Paleontological Statistics Software Package for Education and Data Analysis). Relative abundance, Margalef's species richness, Shannon-Wiener index, Simpson's index, Berger-Parker index and Pielou's evenness index were estimated. For estimating relative abundance, all species of coccinellids collected in the study viz., phytophagous, mycophagous and predaceous beetles were included, while diversity indices were worked out for mycophagous and predaceous beetles.

- **Relative abundance**

Relative abundance is the proportion of individuals of a species in the total population.

$$\mathbf{P_i = (n_i/N) \times 100}$$

P_i = Proportion of individuals found in the i^{th} species (relative abundance)

n_i = Number of individuals belonging to i^{th} species

N = Total number of individuals

- **Margalef's species richness**

Species richness denotes the number of different species represented in an ecological community (Margalef, 1958)

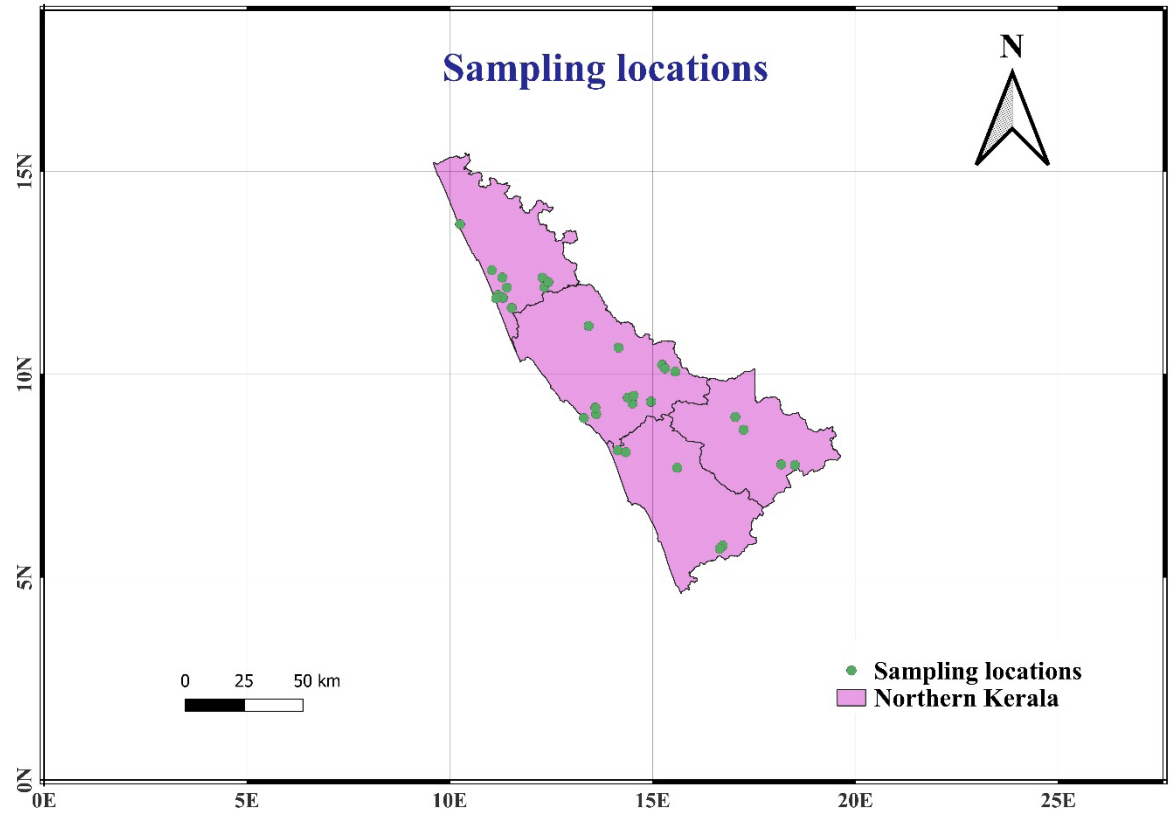


Figure 1. Sampling locations represented using QGIS 3.30.3 software.

d = (S-1)/ ln N

d = Margalef's species richness

S = Number of species

N = Total number of individuals in all the sample

- **Shannon-Wiener index (H)**

The Shannon-Wiener formula was utilised to calculate species diversity (Shannon & Weaver, 1949).

$$H = -\sum_{i=1}^{n} pi \ln pi$$

H = Shannon-Wiener index

Pi = Proportion of individuals found in the i^{th} species

- **Simpson's index (D)**

Simpson's index is a measure of dominance of species (Simpson, 1949).

$D = -\Sigma P_i^2$

Pi= Proportional abundance of i^{th} species (relative abundance)

- **Berger Parker index**

Index of dominance concerning the most abundant species in a community (Berger & Parker, 1970).

BPI = Nmax/ N

Nmax= Number of individuals of the most abundant species

N = Total number of individuals in the community

- **Pielou's evenness index**

Species evenness indicates how evenly species are distributed in the ecosystem (Pielou, 1966).

J=H/(ln(S))

J = Pielou's evenness index

H = Shannon-Wiener index

S = Total number of species

DNA isolation and PCR amplification

The DNA was isolated using the DNASure Tissue Mini Kit (Genetix) following the manufacturer's protocol. The quality and quantity of the DNA isolated were analysed using a Nanodrop spectrophotometer (NanoDrop One – Thermo scientific). Absorbance was recorded at wavelengths of 260 nm and 280 nm, and purity of DNA was indicated by A260/ A280 ratio. The BET buffer in the extraction kit, in which the extracted DNA was stored, was used as blank to record Nanodrop readings.

The isolated DNA was subjected to polymerase chain reaction (PCR) to amplify the 658 bp region near the 5′ terminus of mitochondrial DNA, cytochrome oxidase subunit I (*CO1*), by following standard protocols. The reaction was carried out in 40 µl reaction volume containing 20 µl PCR master mix, 2 µl template DNA, 1 µl forward primer, 1 µl reverse primer, and 16 µl molecular water. The forward and reverse primers used for the reaction were LCO 1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO 2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ respectively (Folmer *et al*., 1994). The PCR was performed using a SimpliAmp Thermal cycler (Thermo Fisher Scientific). Gradient PCR was carried out for standardising the annealing temperature. The best annealing temperature for this reaction was recorded as 51°C. Thermal cycling included an initial denaturation of 94 °C for 4 min, followed by 30 cycles of denaturation of 94°C for 30s, annealing 51°C for 1 min and extension of 72°C for 2 min, followed by a final extension of 72°C for 10 min. Agarose Gel Electrophoresis (AGE) in 1.2 per cent agarose gel was carried out and a Bio-Rad gel documentation system was used to analyse the gel.

Sequencing and molecular analysis

Sanger sequencing of PCR product was done by outsourcing to Biokart India Pvt. Ltd., Bangalore. Bidirectional sequencing was done and trimming and combining of forward and reverse sequences were done using BioEdit Sequence Alignment Editor software and CAP3 sequence assembler. Sequences were checked for stop codons using the Expasy translate tool. Sequence homology analysis was done using NCBI nucleotide BLAST. The sequences generated were submitted to NCBI GenBank and BOLD (Barcode of Life Data Systems).

Phylogenetic analysis

The maximum likelihood tree was constructed using 22 sequences of 18 species generated in this study, along with 19 sequences of these species retrieved from the NCBI database. Phylogenetic analysis was performed using MEGA 11 software with 500 bootstrap iterations. *Amphix laevigatus* (Coleoptera: Endomychidae) was used as an outgroup (Seago *et al*., 2011) (Table 1).

RESULTS

Coccinellids and their prey range

Twenty-seven species belonging to 18 genera of seven tribes *viz*., Aspidimerini, Chilocorini, Coccinellini, Epilachnini, Scymnni, Stethorini, and Sticholotidini of the subfamily Coccinellinae were recorded in the present study. Out of these, three species viz., *Afidenta misera* (Weise), *Henosepilachna septima* (Dieke), and *H. vigintioctopunctata* (Fabricius) were phytophagous and two species were mycophagous *viz*., *Illeis bielawskii* Ghorpade and *Illeis cincta* (Fabricius) and the remaining 22 species were predacious in nature. Predatory coccinellid beetles recorded during the study are *Anegleis cardoni* (Weise), *Brumoides suturalis* (Fabricius), *Cheilomenes sexmaculata* (Fabricius), *Chilocorus nigritus* (Fabricius), *Coccinella septempunctata* (Linnaeus), *Coccinella transversalis* (Fabricius), *Coelophora saucia* (Mulsant), *Cryptogonus kapuri* Ghorpade, *Harmonia octomaculata* (Fabricius), *Jauravia* sp.1, *Jauravia* sp.2, *Micraspis* sp., *Pseudaspidimerus trinotatus* (Thunberg), *Propylea dissecta* (Mulsant), *Scymnus castaneus* group, *Scymnus latemaculatus* Motschulsky, *Scymnus* sp. nr. *posticalis*, *Scymnus saciformis* Motschulsky, *Scymnus* sp.1, *Scymnus* sp.2, *Stethorus* sp., and *Synonychimorpha chittagongi* (Vazirani) (Plates 1-5). The prey and associated host plants of coccinellid beetles observed during this study are given in Table 2.

Species-wise distribution of coccinellid fauna in different agroecological zones

Species-wise distribution of coccinellid fauna in different agroecological zones and cropping systems is given in Table 3. The highest number of 20 species were reported from high hills (rice - 4 species; vegetables -10 species; plantation crops - 5 species and fruit crops - 6 species). A total of 19 species of coccinellids were recorded from the coastal region. Of these, three species were recorded from rice, 14 species from vegetables, seven species from plantation crops and four species from fruit crops. Fourteen species were collected from the midland laterites zone (rice - 4 species; vegetables - 8 species; plantation crops - 3 species and fruit crops - 3 species). Only ten species of ladybirds were recorded in the foothills, four species from rice, three from vegetables, three from plantation crops and three from fruit crops. Among the different crops surveyed from different zones, the highest number of coccinellid species were recorded from vegetables (18), followed by plantation crops (11), fruit crops (8) and rice (7).

Estimation of diversity indices

Relative abundance

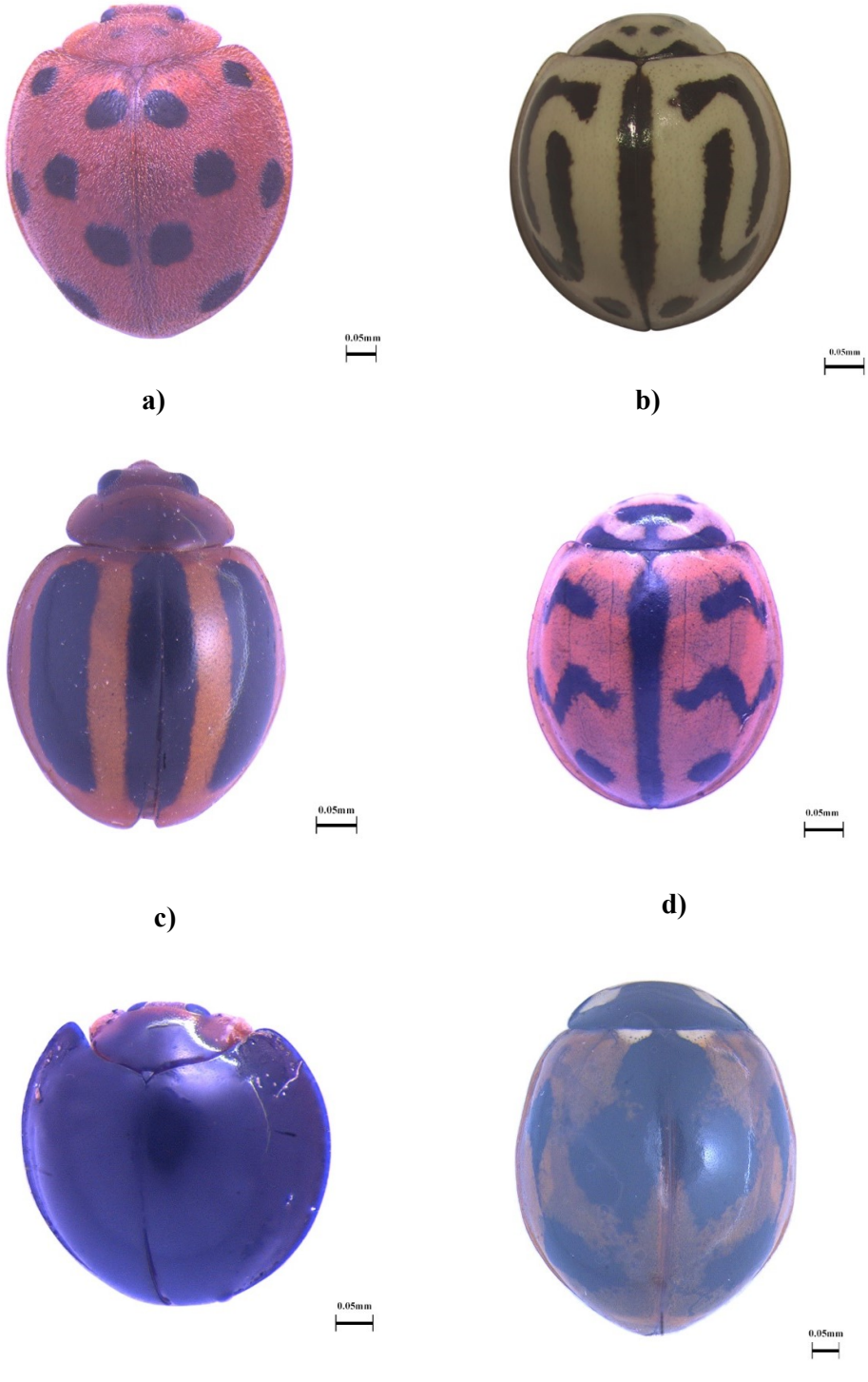
The relative abundance of coccinellid fauna in different agro ecological zones and cropping systems are given in

Table 1. NCBI accession numbers of sequences retrieved from NCBI for phylogenetic analysis

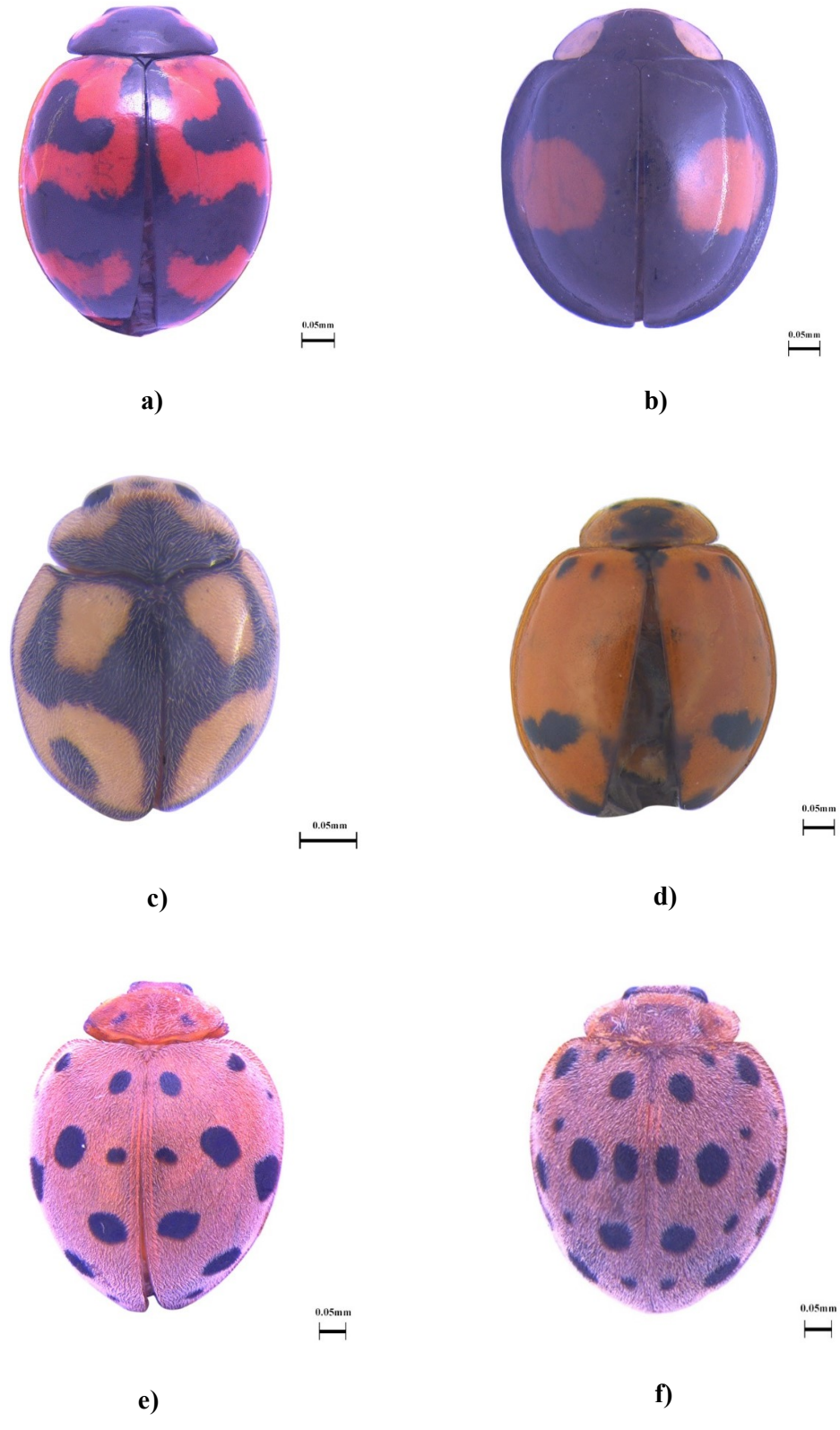
Species	NCBI accession number
Scymnus castaneus	ON703377
Scymnus latemaculatus	ON980762
Synonychimorpha chittagongi	ON738450
Henosepilachna vigintioctopunctata	OM760638
	OM760635
Henosepilachna septima	MW221939
	KT693136
Coelophora (=*Lemnia*) *saucia*	MK574678
	MN053056
	MN206422
Coccinella transversalis	KT693133
	KT175577
Harmonia octomaculata	ON568150
Cheilomenes sexmaculata	MZ334467
	KP851143
Micraspis sp.	KF958279
Anegleis cardoni	JN128609
Illeis cincta	MZ047080
	KY694472
Amphix laevigatus (Outgroup)	EU164679

Table 2. Host plants and associated prey species of coccinellid beetles

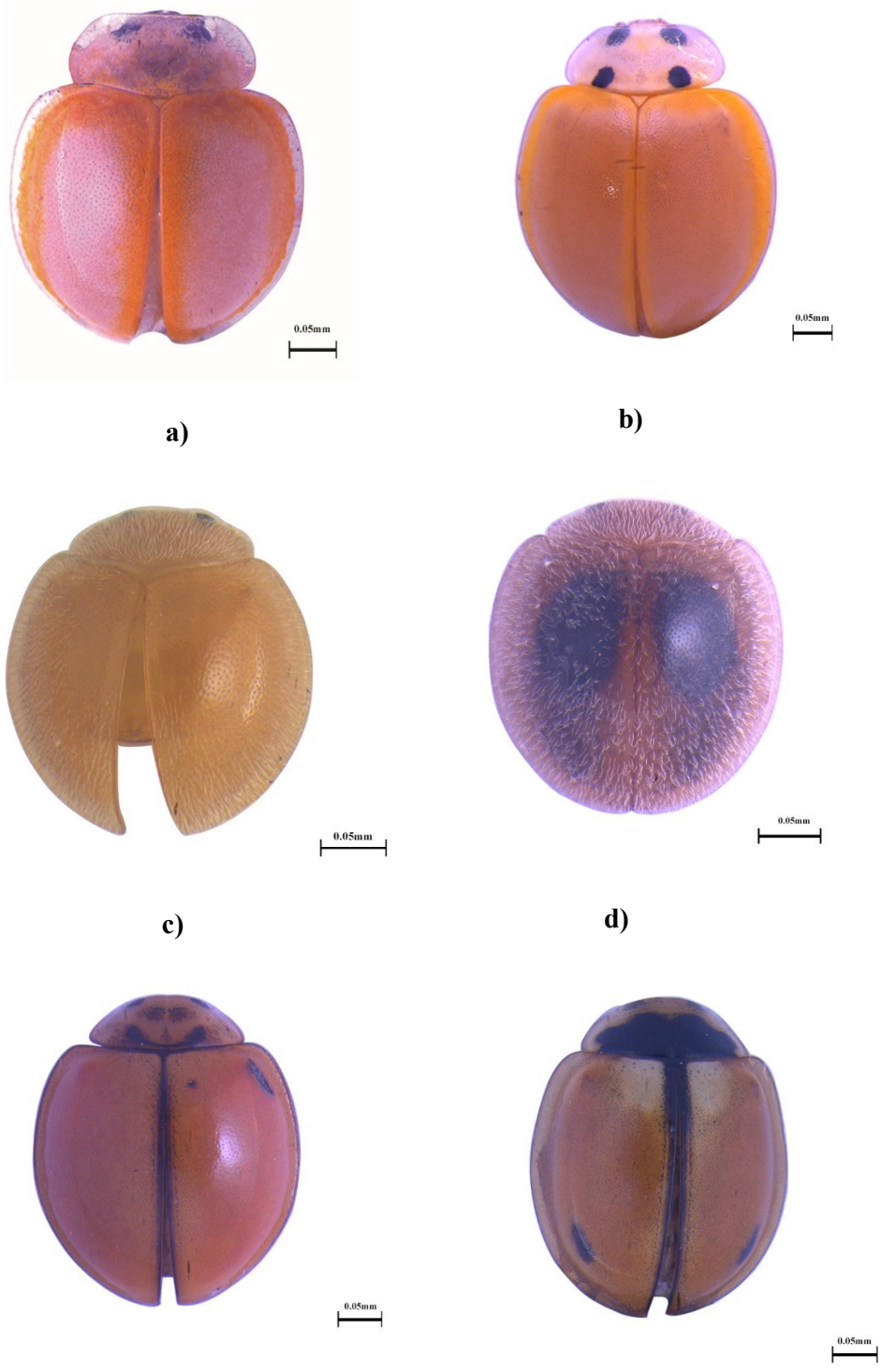
Sl. No.	Species	Host plant	Prey/ Fungus
1	*Afidenta misera*	Cowpea	-
2	*Anegleis cardoni*	Cowpea, siam weed	*Aphis craccivora*
3	*Brumoides suturalis*	Brinjal, chilli, rice	*Aphis gossypii*
4	*Cheilomenes sexmaculata*	Brinjal, cowpea, cabbage	*A. craccivora, A. gossypii, Myzus persicae,*
5	*Chilocorus nigritus*	Coconut	*Aspidiotus destructor*
6	*Coccinella septempunctata*	Bitter gourd	Not known
7	*Coccinella transversalis*	Brinjal, bitter gourd, chilli, cowpea, okra, rice	*A. craccivora, A. gossypii*
8	*Coelophora saucia*	Cowpea, rice	Aphid
9	*Cryptogonus kapuri*	Areca nut	Not known
10	*Harmonia octomaculata*	Rice	Not known
11	*Henosepilachna septima*	Bitter gourd, ridge gourd, snake gourd	-
12	*Henosepilachna vigintioctopunctata*	Brinjal	-
13	*Illeis bielawskii*	Ridge gourd	Powdery mildew
14	*Illeis cincta*	Bitter gourd, ridge gourd	Powdery mildew
15	*Jauravia* sp.1	Areca nut, coconut, cowpea, guava, jackfruit, peanut butter fruit, ridge gourd	*A. craccivora,* mealybug, scale insects, whitefly
16	*Jauravia* sp.2	Avocado	Mealybug
17	*Micraspis* sp.	Rice	Not known
18	*Propylea dissecta*	Cowpea, okra, rice	*A. craccivora*
19	*Pseudaspidimerus trinotatus*	Areca nut, avocado, Siam weed, coconut, cowpea, gliricidia, guava, jackfruit	*A. craccivora,* mealybug, whitefly
20	*Scymnus castaneus* group	Chilli, cowpea, jack fruit	Aphid, mealybug, scale insect
21	*Scymnus latemaculatus*	Siam weed	*A. craccivora,*
22	*Scymnus* sp. *nr. posticalis*	Chilli, siam weed, tea	*A. craccivora, Toxoptera aurantii*
23	*Scymnus saciformis*	Peanut butter fruit	Not known
24	*Scymnus* sp.1	Areca nut	Not known
25	*Scymnus* sp.2	Coffee, guava, okra, papaya, peanut butter fruit	Mealybug, scale insects
26	*Stethorus* sp.	Areca nut, jackfruit,	Tetranychid mite
27	*Synonychimorpha chittagongi*	Guava, jackfruit	*Greenidea psidii*, mealybug, scale insect, whitefly



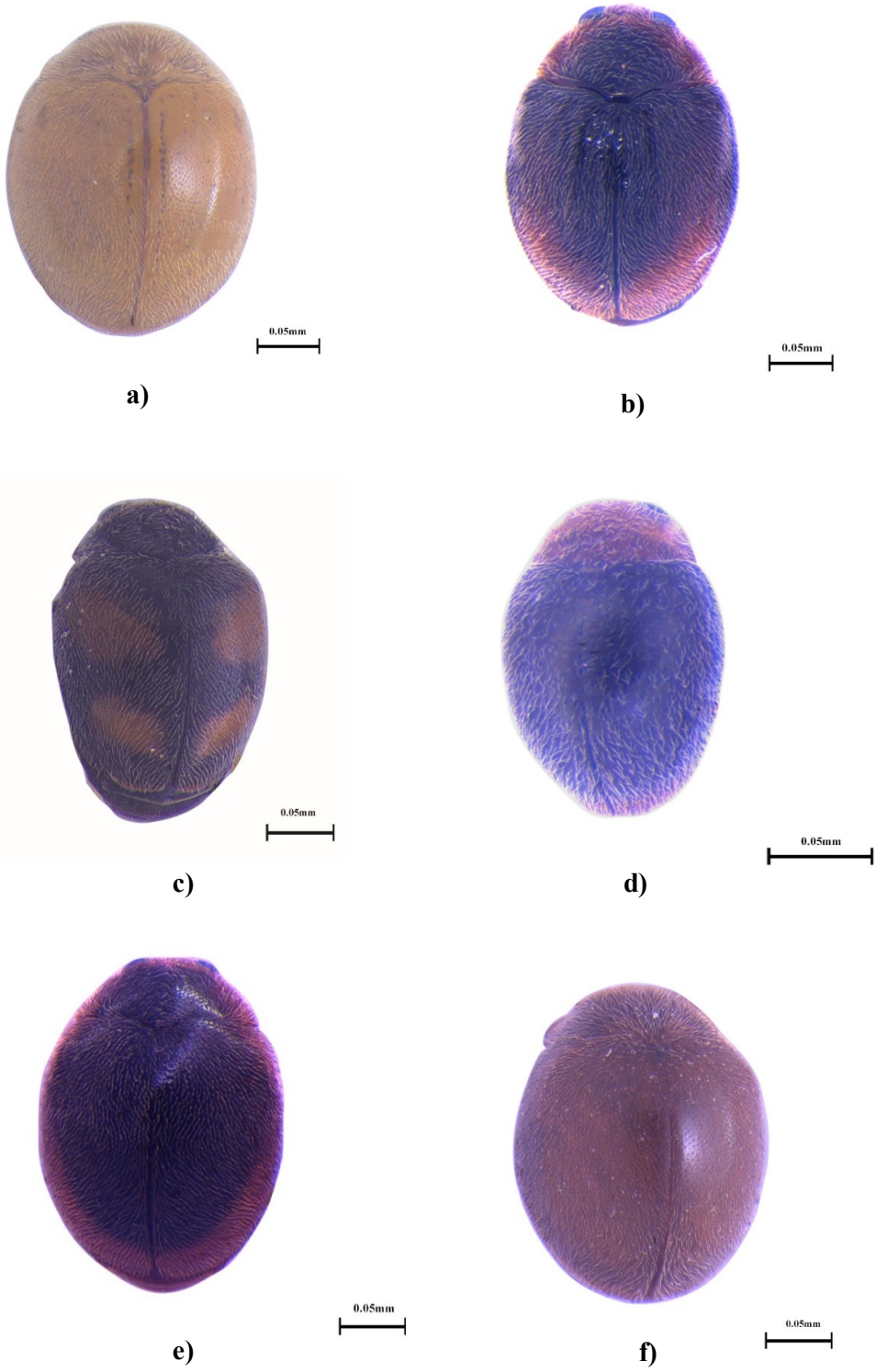
(a) *Afidenta misera* **(b)** *Anegleis cardoni* **(c)** *Brumoides suturalis*
(d) *Cheilomenes sexmaculata* **(e)** *Chilocorus nigritus* **(f)** *Coccinella septempunctata*
Plate 1. Coccinellid beetles collected during the study.



(a) *Coccinella transversalis* **(b)** *Coelophora saucia* **(c)** *Cryptogonus kapuri*
(d) *Harmonia octomaculata* **(e)** *Henosepilachna septima* **(f)** *Henosepilachnavigintioctopunctata*
Plate 2. Coccinellid beetles collected during the study.

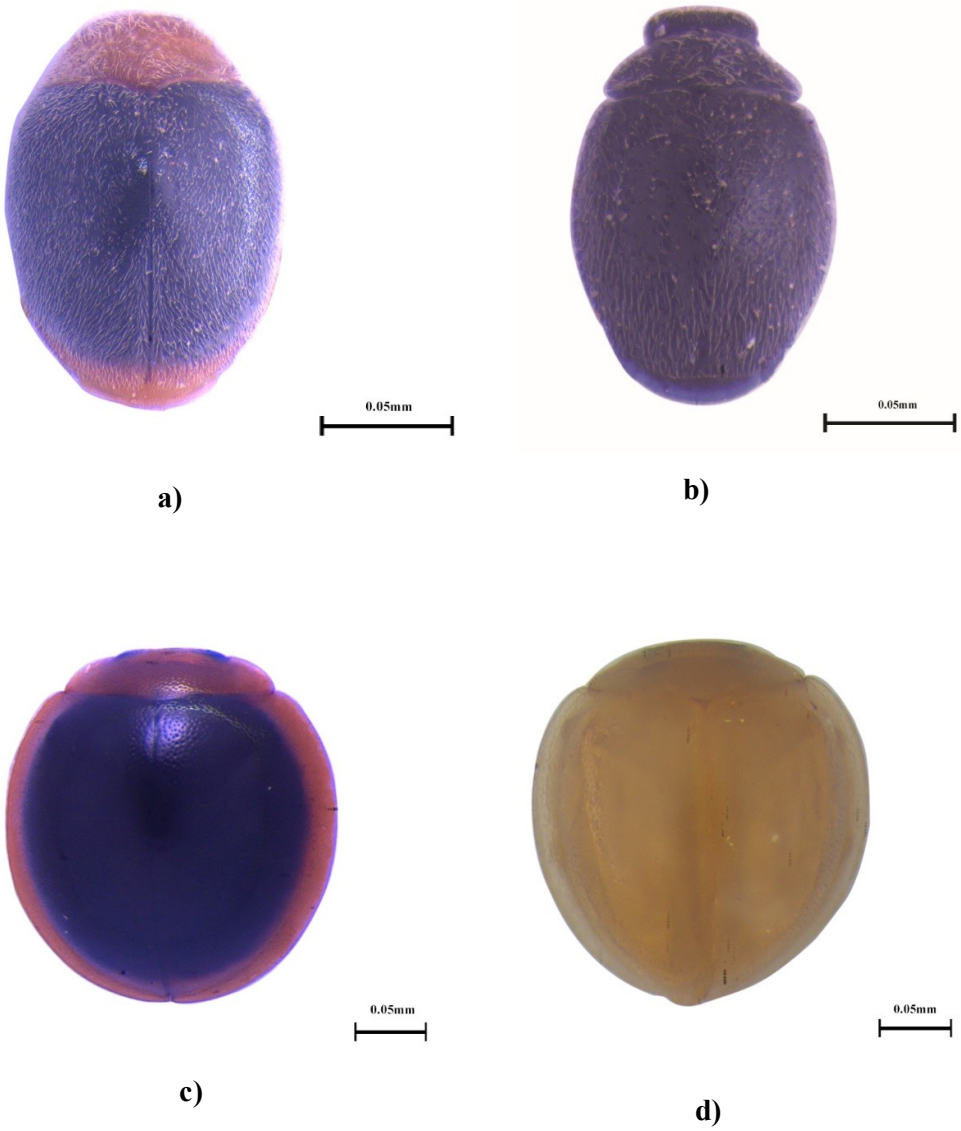


(a) *Illeis bielawskii* **(b)** *Illeis cincta* **(c)** *Jauravia sp.1* **(d)** *Jauravia sp.2*
(e) *Micraspis sp.* **(f)** *Propylea dissecta*
Plate 3. Coccinellid beetles collected during the study.



(a) *Pseudaspidimerus trinotatus* **(b)** *Scymnus castaneus group* **(c)** *Scymnus latemaculatus* **(d)** *Scymnus nr. posticalis* **(e)** *Scymnus saciformis* **(f)** *Scymnus sp.1*

Plate 4. Coccinellid beetles collected during the study.



(a) *Scymnus sp.2* **(b)** *Stethorus sp.* **(c)** *Synonychimo rpha chittagongi - Morphotype 1*
(d) *Synonychimorpha chittagongi - Morphotype 2*
Plate 5. Coccinellid beetles collected during the study.

Table 4. Irrespective of the zones and crops surveyed, the most abundant coccinellid species in northern Kerala was *H. vigintioctopunctata* (29.86%). Among the predatory coccinellids, the most abundant species was *C. sexmaculata* (11.88%), followed by *C. transversalis* (11.72%).

The most abundant lady beetle in the coastal region and midland laterites were *H. vigintioctopunctata* (41.81%) and *A. misera* (18.82%), respectively. Among the predatory coccinellids, *C. sexmaculata* and *C. transversalis* were the abundant species in coastal plains, midland laterites and foot hills. *Coelophora saucia* (20.93%) was recorded as the most abundant predatory coccinellid in hilly region.

In rice fields, the most abundant species was *C. transversalis* (65.45%), followed by *Micraspis* sp. (16.36%). In the vegetable fields, the phytophagous species, *H. vigintioctopunctata* (42.76%) was found to be the most abundant coccinellid species followed by the predatory coccinellid, *C. sexmaculata* (17.01%). *Chilocorus nigritus* was the most abundant lady beetle in plantation crops, with a relative abundance of 25.64 per cent, followed by *P. trinotatus* (20.51%) and *Jauravia* sp.1 (19.23%). In fruit crops, *P. trinotatus* (34.55%) recorded the highest abundance, followed by *S. chittagongi* (23.64%) and *Jauravia* sp.1 (23.64%).

Margalef's species richness (d)

The total species richness of predatory coccinellid fauna in this study was found to be 3.88 (Table 5). Among the crops, the vegetable (2.67) cropping system was the most species-rich, whereas rice (1.50) ecosystem was the least rich. Across

Table 3. Species wise distribution of coccinellid fauna in different crops in different agroecological zones

Agroecological zone	Cropping system	Coccinellid species
Coastal plain	Rice	*B. suturalis*, *C. transversalis*, *P. trinotatus*
	Vegetables	*A. misera*, *A. cardoni*, *B. suturalis*, *C. sexmaculata*, *C. transversalis*, *H. octomaculata*, *H. septima*, *H. vigintioctopunctata*, *I. bielawskii*, *Jauravia* sp.1, *P. dissecta, P. trinotatus, S. castaneus* group, *S.* sp. nr. *posticalis*
	Plantation crops	*C. nigritus*, *Jauravia* sp.1, *P. trinotatus, S. castaneus* group, *S. latemaculatus*, *S.* sp. nr. *posticalis, Stethorus* sp.
	Fruit crops	*Jauravia* sp.1, *P. trinotatus, Scymnus* sp.2, *S. chittagongi*
Midland laterites	Rice	*B. suturalis*, *C. transversalis*, *Micraspis* sp., *P. dissecta*
	Vegetables	*A. misera*, *C. sexmaculata*, *H. vigintioctopunctata*, *I. cincta*, *Jauravia* sp.1, *P. dissecta, P. trinotatus, S. castaneus* group,
	Plantation crops	*Jauravia* sp.1, *P. trinotatus, Scymnus* sp.1
	Fruit crops	*Jauravia* sp.1, *S. saciformis*, *S. chittagongi*
Foothills	Rice	*B. suturalis*, *C. transversalis*, *H. octomaculata*, *Micraspis* sp.,
	Vegetables	*H. septima*, *P. trinotatus, Scymnus* sp.2
	Plantation crops	*C. kapuri*, *Jauravia* sp.1, *P. trinotatus*
	Fruit crops	*P. trinotatus, Scymnus* sp.2, *S. chittagongi*
High hills	Rice	*C. transversalis*, *C. saucia*, *H. octomaculata*, *P. dissecta*
	Vegetables	*A. cardoni*, *B. suturalis*, *C. sexmaculata*, *C. septempunctata*, *C. transversalis*, *C. saucia*, *H. septima*, *H. vigintioctopunctata*, *I. cincta*, *P. dissecta*
	Plantation crops	*C. kapuri*, *S.* sp. nr. *posticalis*, *Scymnus* sp.2, *Stethorus* sp., *S. chittagongi*
	Fruit crops	*Jauravia* sp.1, *Jauravia* sp.2, *P. trinotatus, S. castaneus* group, *Stethorus* sp., *S. chittagongi*

four agroecological zones, the high hills (3.87) showed the highest species richness, whereas Margalef's species richness index was least in foothills (2.15).

- **Shannon-Wiener index (H)**

The total species diversity of coccinellid fauna recorded in northern Kerala was 2.57. Among the different crops studied, vegetables (1.98) recorded the highest value of Shannon index, which indicates high diversity, followed by plantation crops (1.96) while the rice (1.15) ecosystem recorded the least diversity. Among the agroecological zones, the high hills (2.35) were the most diverse, and the foothills was the least diverse zone (1.81).

- **Simpson's index (D)**

Simpson's index (D) is a measure of dominance of various species in a community. Higher value of Simpson's index indicates the dominance of a few species and less diversity. It can be also expressed as Simpson's index of diversity (1-D) and Simpson's reciprocal index (1/D). Simpson's index of diversity and Simpson's reciprocal index value increases with increasing diversity.

Higher value of Simpson's index (0.46) and lowest values of Simpson's Index of Diversity (0.54) and Simpson's reciprocal index (2.17) were recorded in rice compared to other cropping systems. Among the crop ecosystems, plantation crops (0.17) was found as the most diverse ecosystem. Among the agroecological zones, midland laterites and high hills were observed with highest diversity (Simpson's index- 0.13), while foothills recorded the lowest diversity (Simpson index-0.21).

- **Berger Parker index**

Among different cropping systems, higher dominance was observed in rice (0.65) and lesser dominance in plantation crops (0.26).

Across different agroecological zones, the foothills (0.34) showed the highest dominance and midland laterites (0.19) showed the least dominance.

- **Pielou's evenness index (J)**

The species evenness of the total coccinellid fauna studied was 0.55. The plantation crop (0.65) ecosystem showed the highest species evenness, and the rice (0.45) cropping system showed the lowest evenness value. Among the agroecological zones, the highest species evenness was recorded in the midland laterites (0.77), while the coastal plains (0.57) recorded the least value for evenness.

Table 4. Relative abundance (%) of coccinellid fauna in different agroecological zones and cropping systems

Sl. No.	Species name	Total	Agro-ecological zones				Cropping systems			
			Coastal plains	**Midland laterites**	**Foothills**	**High hills**	**Rice**	**Vegetables**	**Plantation crops**	**Fruit crops**
1	*Afidenta misera*	5.14	3.91	18.82	0.00	0.00	0.00	7.36	0.00	0.00
2	*Anegleis cardoni*	0.64	0.73	0.00	0.00	1.16	0.00	0.92	0.00	0.00
3	*Brumoides suturalis*	1.12	0.49	2.35	2.33	2.33	7.27	0.69	0.00	0.00
4	*Cheilomenes sexmaculata*	11.88	13.69	4.71	0.00	16.28	0.00	17.01	0.00	0.00
5	*Chilocorus nigritus*	3.21	4.89	0.00	0.00	0.00	0.00	0.00	25.64	0.00
6	*Coccinella septempunctata*	0.32	0.00	0.00	0.00	2.33	0.00	0.46	0.00	0.00
7	*Coccinella transversalis*	11.72	7.82	12.94	32.56	18.60	65.45	8.51	0.00	0.00
8	*Coelophora saucia*	2.89	0.00	0.00	0.00	20.93	1.82	3.91	0.00	0.00
9	*Cryptogonus kapuri*	0.48	0.00	0.00	4.65	1.16	0.00	0.00	3.85	0.00
10	*Harmonia octomaculata*	0.64	0.49	0.00	2.33	1.16	3.64	0.46	0.00	0.00
11	*Henosepilachna septima*	4.65	6.11	0.00	4.65	2.33	0.00	6.67	0.00	0.00
12	*Henosepilachna vigintioctopunctata*	29.86	41.81	14.12	0.00	3.49	0.00	42.76	0.00	0.00
13	*Illeis bielawskii*	2.25	3.42	0.00	0.00	0.00	0.00	3.22	0.00	0.00
14	*Illeis cincta*	0.80	0.00	2.35	0.00	3.49	0.00	1.15	0.00	0.00
15	*Jauravia* sp.1	4.82	2.44	11.76	11.63	5.81	0.00	0.46	19.23	23.64
16	*Jauravia* sp.2	0.16	0.00	0.00	0.00	1.16	0.00	0.00	0.00	1.82
17	*Micraspis* sp.	1.44	0.00	5.88	9.30	0	16.36	0.00	0.00	0.00
18	*Propylea dissecta*	1.12	0.73	2.35	0.00	2.33	3.64	1.15	0.00	0.00
19	*Pseudaspidimerus trinotatus*	7.22	5.87	9.41	23.26	3.49	1.82	2.07	20.51	34.55
20	*Scymnus castaneus* group	2.09	2.44	1.18	0.00	2.33	0.00	2.30	1.28	3.64
21	*Scymnus latemaculatus*	0.64	0.98	0.00	0.00	0.00	0.00	0.00	5.13	0.00
22	*Scymnus* sp. nr. *posticalis*	2.25	3.18	0.00	0.00	1.16	0.00	0.69	14.10	0.00
23	*Scymnus saciformis*	0.48	0.00	3.53	0.00	0.00	0.00	0.00	0.00	5.45
24	*Scymnus* sp.1	0.16	0.00	1.18	0.00	0.00	0.00	0.00	1.28	0.00
25	*Scymnus* sp.2	0.80	0.24	0.00	6.98	1.16	0.00	0.23	1.28	5.45
26	*Stethorus* sp.	0.64	0.49	0.00	0.00	2.33	0.00	0.00	3.85	1.82
27	*Synonychimorpha chittagongi*	2.57	0.24	9.41	2.33	6.98	0.00	0.00	3.85	23.64

Sequence analysis and DNA barcoding

A total of 22 sequences of 18 species were generated in this study. Of the 22 sequences, 14 DNA sequences (13 species viz., *A. cardoni, C. sexmaculata, C. transversalis, C. saucia, H. octomaculata, H. septima, H. vigintioctopunctata, I. cincta, Micraspis* sp. *Propylea* sp., *S. castaneus* (2 accessions)*, S. chittagongi*, and *S. latemaculatus* showed more than 98 per cent similarity with sequences in the NCBI database and confirmed their identity. Eight sequences of five species (*Cryptogonus kapuri* (one sequence), *Illeis bielawskii* (two sequences), *Jauravia* sp. (two sequences), *P. trinotatus* (one sequence), *S.* sp. nr. *posticalis* (two sequences)) showed less than 90 % similarity to other sequences in the NCBI database. The 22 sequences generated during the study were submitted to the NCBI GenBank and BOLD and details of NCBI accession number are given in Table 6.

Phylogenetic tree

Phylogenetic tree constructed using MEGA11 software is given in Figure 2. The phylogenetic tree formed distinct clades. *Coelophora saucia* and *P. dissecta* formed a single clade and all other species formed second major clade. All tribes formed separate clades except for Aspidimerini and Scymnini. *Scymnus* sp. nr. *posticalis* showed higher affinity towards tribe Aspidimerini than rest of the members of tribe Scymnini.

DISCUSSION

Diversity of coccinellids

Family Coccinellidae includes three subfamilies *viz*., Microweiseinae, Coccinellinae and Monochorinae. Coccinellinae is the largest subfamily with 26 tribes

Table 5. Diversity indices for predatory coccinellid fauna in different agroecological zones

Agroecological zone	Cropping system	Margalef's species richness (d)	Shannon-Wiener index (H)	Simpson's index, (D)	Simpson's Index of Diversity (1-D)	Simson's reciprocal index (1/D)	Berger Parker index	Species evenness (J)
Coastal plain	Rice	1.24	0.95	0.44	0.56	2.27	0.60	0.86
	Vegetables	2.07	1.66	0.28	0.72	3.57	0.45	0.48
	Plantation crops	1.55	1.61	0.25	0.75	4.00	0.42	0.71
	Fruit crops	1.02	0.90	0.52	0.48	1.92	0.68	0.61
Total (Coastal plain)		**2.84**	**2.21**	**0.15**	**0.85**	**6.67**	**0.28**	**0.57**
Midland laterites	Rice	1.02	1.06	0.42	0.52	2.38	0.58	0.72
	Vegetables	1.95	1.61	0.23	0.77	4.35	0.31	0.83
	Plantation crops	0.87	0.94	0.42	0.58	2.38	0.50	0.86
	Fruit crops	0.74	1.01	0.40	0.60	2.5	0.53	0.91
Total (Midland laterites)		**2.72**	**2.22**	**0.13**	**0.87**	**7.69**	**0.19**	**0.77**
Foothills	Rice	1.00	0.87	0.54	0.46	1.85	0.70	0.60
	Vegetables	1.44	0.69	0.50	0.50	2.00	0.50	1.00
	Plantation crops	0.78	1.01	0.38	0.62	2.63	0.46	0.92
	Fruit crops	1.12	1.01	0.39	0.61	2.56	0.50	0.92
Total (Foothills)		**2.15**	**1.81**	**0.21**	**0.79**	**4.76**	**0.34**	**0.68**
High hills	Rice	1.25	0.89	0.55	0.45	1.81	0.73	0.61
	Vegetables	1.81	1.63	0.25	0.75	4.00	0.35	0.63
	Plantation crops	2.06	1.48	0.27	0.73	3.70	0.43	0.87
	Fruit crops	1.85	1.64	0.22	0.78	4.55	0.33	0.86
Total (High hills)		**3.87**	**2.35**	**0.13**	**0.87**	**7.69**	**0.22**	**0.58**
Total	Rice	1.50	1.15	0.46	0.54	2.17	0.65	0.45
	Vegetables	2.67	1.98	0.22	0.78	4.55	0.39	0.48
	Plantation crops	2.30	1.96	0.17	0.83	5.88	0.26	0.65
	Fruit crops	1.75	1.63	0.24	0.76	4.17	0.34	0.64
Total		**3.88**	**2.57**	**0.11**	**0.89**	**9.09**	**0.20**	**0.55**

(Che *et al*., 2021). In this study, twenty-seven species belonging to 18 genera in seven tribes viz., Aspidimerini, Chilocorini, Coccinellini, Epilachnini, Scymnini, Stethorini and Sticholotidini of the subfamily Coccinellinae were identified from different agroecological zones of northern Kerala. Poorani (2002, 2012) reviewed and listed 479 species of coccinellids of different subfamilies from Indian subcontinent. Later, Janakiraman & Thangjam (2019), added 14 species to the known fauna of coccinellids of India. Among the coccinellids collected during the study, Coccinellini (10 species) was the predominant tribe, followed by tribe Scymnini (6 species). These tribes together contributed 59 per cent of total coccinellid fauna collected from northern Kerala. According to Poorani (2002) subfamily Coccinellinae (now under the tribe Coccinellini) and Scymninae (now under the tribe Scymnini) together forms 63.75 per cent of total coccinellid fauna of Indian subcontinent.

Among the different crops studied, rice ecosystem was found to be the least species-rich, least diverse, and less evenly distributed. Only seven species were recorded from rice among which these two species, viz., *Coccinella transversalis* and *Micraspis* sp. alone contributes 81.81 per cent abundance. During field surveys, the two species were also found on the flowering plants along the bunds and on weed hosts in the un-weeded rice fields. These two species have also been reported as pollen feeders (Shanker *et al*., 2013). These alternate food sources might have enabled them to dominate in rice fields. The over dominance of these two species might have resulted in lesser diversity and less even distribution of coccinellid fauna in rice.

Highest species richness and diversity were observed in vegetables. *H. vigintioctopunctata* was the most abundant coccinellid species. There was severe infestation of *H. vigintioctopunctata* on brinjal in coastal plains, that reflected in the relative abundance of this species. However, among predatory coccinellids, *C. sexmaculata* followed by *C.*

Table 6. NCBI accession numbers of sequences submitted

Sl. No	Species	NCBI Accession number
1	*Anegleis cardoni*	OR405498
2	*Cheilomenes sexmaculata*	OR405117
3	*Coccinella transversalis*	OR405118
4	*Coelophora saucia*	OR405105
5	*Cryptogonus kapuri*	OR416856
6	*Harmonia octomaculata*	OR405123
7	*Henosepilachna septima*	OR405125
8	*Henosepilachna vigintioctopunctata*	OR405121
9	*Illeis bielawskii*	OR990595
10	*Illeis bielawskii*	OR990594
11	*Illeis cincta*	OR405127
12	*Jauravia* sp.1	OR416935
13	*Jauravia* sp.1	OR416855
14	*Micraspis* sp.	OR416861
15	*Propylea dissecta*	OR405160
16	*Pseudaspidimerus trinotatus*	OR423068
17	*Scymnus castaneus*	OR405307
18	*Scymnus castaneus*	OR405310
19	*Scymnus latemaculatus*	OR405488
20	*Scymnus* sp. nr. *posticalis*	OR416858
21	*Scymnus* sp. nr. *posticalis*	OR416857
22	*Synonychimorpha chittagongi*	OR405376

transversalis were the dominant species. Aphids were the major sucking pests associated with vegetable crops surveyed during the study, and hence could justify the abundance of these two aphidophagous coccinellids in vegetable fields. Several authors also reported *C. sexmaculata* and *C. transversalis* as the most abundant species in vegetable ecosystem (Rekha *et al*., 2009; Murali *et al.*, 2017; Borkakati *et al*., 2019; Sruthi *et al*., 2021).

Diversity in terms of Simpson's indices, Berger Parker index and species evenness were more in plantation crops. *C. nigritus* was the most dominant lady beetle in plantation crops, followed by *P. trinotatus* and *Jauravia* sp.1. Severe infestation of the scale insect, *Aspidiotus destructor* was recorded in two coconut plantations surveyed in the coastal region. Kinawy (1991) reported *C. nigritus* as a good predator of *Aspidiotus destructor* in coconut. The abundance of prey may be the reason behind the higher abundance of *C. nigritus* in coconut. *P. trinotatus* recorded the highest abundance in the fruit crops, followed by *S. chittagongi*. These two species are known to prey upon mealybugs and aphids (Omkar & Pervez, 2004). Aphids and mealybugs were recorded in the fruit crops during the study.

Among the different agroecological zones of northern Kerala studied, high hills were found to be the most diverse and species rich agroecological zone, with respect to coccinellid fauna. But species were more evenly distributed in midland laterites. Western Ghats lying in high hills of northern Kerala would have contributed to the higher diversity of cocinellid species. Western Ghats are renowned for their remarkable biological diversity and endemism. It is recognized as one of the world's eight 'hottest hotspots' of biological diversity (UNESCO, 2016; Poorani & Booth, 2017). In the present study, a highest number of 18 species were reported from high hills.

CONCLUSION

In the present study, 27 coccinellid species belonging to 18 genera of seven tribes with their associated prey

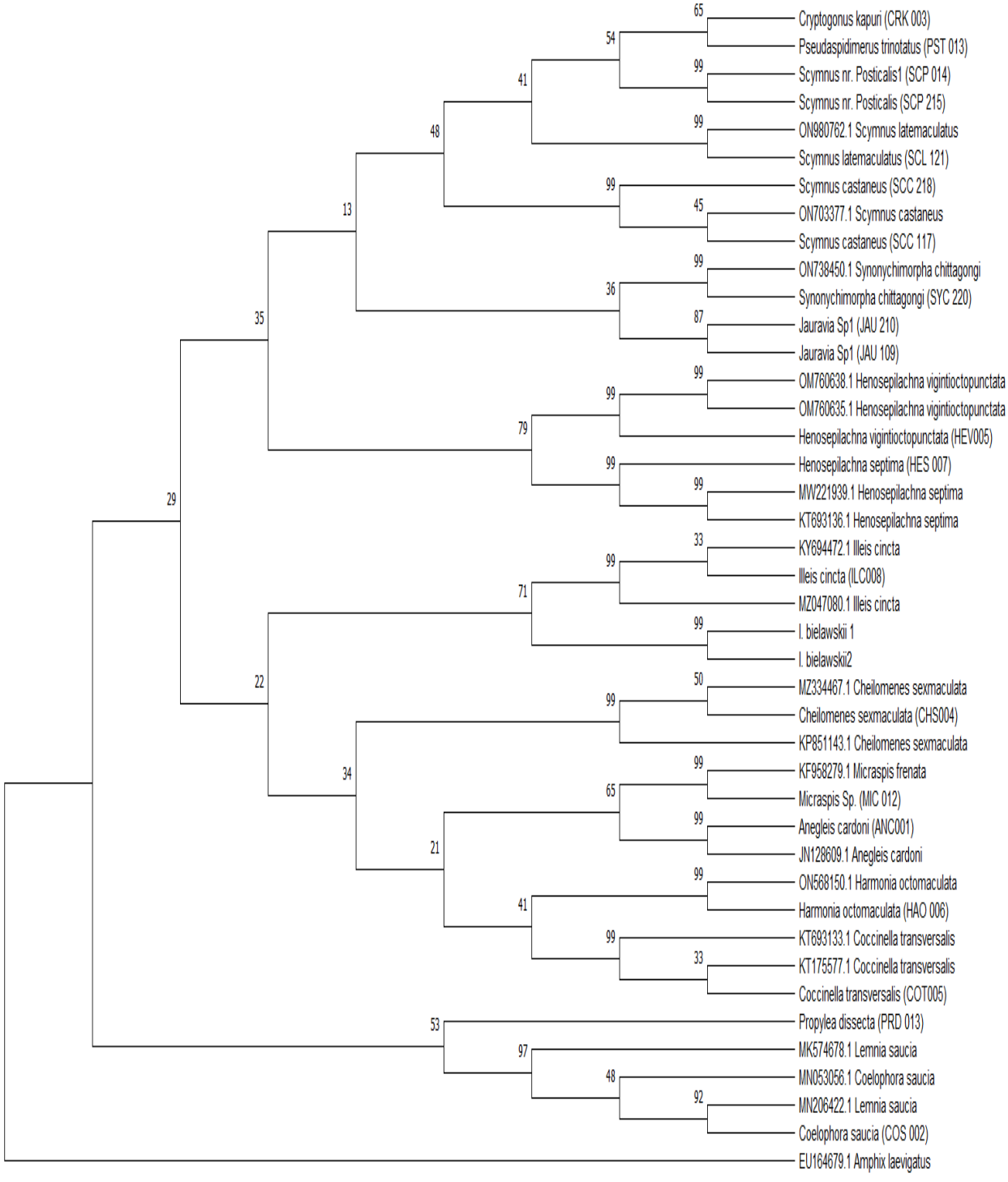


Figure 2. Maximum likelihood based phylogenetic tree of coccinellid beetles collected during the study.

and host plants are documented. Tribe Coccinellini was recorded as the predominant tribe. High hills was found to be the most diverse agroecological zone for Coccinellidae in northern Kerala, whereas planation crops and vegetables were the most diverse ecosystems exhibiting high coccinellid diversity. Twenty-two mitochondrial cytochrome c oxidase 1 (COI) sequences of 18 species were submitted to NCBI and BOLD, which could be used as reference for further studies. Among these, eight sequences of 5 species were new addition to NCBI database.

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