AATCC Review

23 November 2022: Received
11 March 2023: Revised
15 May 2023: Accepted
02 June 2023: Available Online

www.aatcc.peerjournals.net

Research Article Open Access

DNA Barcoding and Phylogenetic Analysis of Ladybird Beetles from South Gujarat, India

Mangali Ashwini and Abhishek Shukla*

Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, India

ABSTRACT

The diversity of the family Coccinellidae is of great practical and scientific importance due to their worldwide utilization as natural enemies of phytophagous insects. The COI gene sequences of ten coccinellid species were generated and submitted to NCBI database with accession numbers. The phylogenetic analysis using molecular data revealed that the family Coccinellidae was monophyletic in origin. The species of tribe Coccinellini formed a single clade and had shown monophyly. The tribe Epilachnini and Stethorini formed the basal clades. The interspecific genetic distance among the Coccinellid species ranged between 0.170–0.315 with the lowest genetic distance of 0.170 between C. transversalis and P. bisoctonotata while the highest genetic distance was found between H. octomaculata and S. pauperculus (0.315). This study confirms the monophyletic origin of the family Coccinellidae.

Keywords: *DNA barcoding, phylogenetics, coccinellidae*

INTRODUCTION

Gujarat is the microcosm of India and displays considerable heterogeneity in terms of agro-meteorological and climatic conditions [1]. This supports the good growth of crops and is favorable for the development of sucking pests. Coccinellids are known to have a close association with these sucking pests. The utilization of coccinellids in biological control has been a roller coaster ride since its first successful attempt to control cotton cushiony scale,Iceryapurchasi (Maskell) by vedalia beetle,Novius (Rodolia) cardinalis(Mulsant).

The Coccinellid fauna of the world consists of 6000 species in 360 genera, two subfamilies and 30 tribes [2]. The coccinellid fauna of India is diverse and rich, but it is poorly known in comparison to that of other areas of the world. About 550 species are known from the Indian subcontinent, grouped into 90 genera, 16 tribes, and two subfamilies, with hundreds of undescribed species in prominent tribes such as Scymnini and Sticholotidini, etc.[3].

Coccinellids are known to occur in different agroecosystems where both the adult and larval stages are feeding on various crop pests. Their diversity is of great practical and scientific importance due to their worldwide utilization as natural enemies of phytophagous insects. The color pattern on the elytra of coccinellids is quite variable, even within species. This often leads to confusion and misidentification of coccinellids, thereby posing a challenging task for taxonomists and phylogenetics. The traditional taxonomic identification of ladybird beetles has always relied on morphological features [4]. However, it is difficult and time-consuming to accurately identify based on morphological criteria, as a large number of ladybird beetles exhibit polymorphism.

Hebert et al. [5] proposed a new technique for taxon identification by exploiting the diversity among DNA sequences of organisms called 'DNA Barcoding'. The 5' region of the mitochondrial cytochrome c oxidase subunit I (COI) with a length of 658 base pair has been proposed as a standard barcode for animals [6]. The universal primers for this gene are robust, allowing recovery of its 5' end from members of most animal phyla and COI appears to have a greater range of phylogenetic signals than any other mitochondrial gene. A mean intraspecific divergence of 10 times was proposed as the standard threshold for differentiating species [5]. Since the initial use of DNA barcoding for insect identification in 2003, DNA barcoding has been used to identify a wide range of insect orders. Presently around 6,585,185 barcodes representing 231,648 insect species have been generated [7]. DNA barcoding is not only valuable for identifying and discovering species in wide-ranging taxa but also suitable for reconstructing phylogenetic relationships among taxa of different phylogenetic ranks [8 and 9]. Despite the fact that barcoding research has been conducted on a variety of Coleopteran beetles [10,11 and 12] (Pentinsaariet al., 2014; Oba et al., 2015; Raupachet al., 2016), there are only a few studies on ladybird beetles, especially from India.

MATERIALS AND METHODS

Sampling

The different species of coccinellids were collected from different habitats of Navsari Agricultural University,

*Corresponding Author: Abhishek Shukla
Email Address: abhishekshukla@nau.in

DOI: https://doi.org/10.58321/AATCCReview.2023.11.03.189

Navsariduring 2021-2022. The samples were preserved in 90 percent ethanol and stored at -20°C. They were further used for DNA isolation, amplification, and sequencing.

DNA extraction

Genomic DNA was extracted from the samples using DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. The elytra and wings of the Coccinellids were removed for sample preparation and DNA extraction.

PCR Protocol

The extracted DNA was used for the amplification of the partial mitochondrial cytochrome oxidase I (COI) gene using polymerase chain reaction (PCR). The COI gene was amplified using the primers LCO and HCO using an initial denaturation step of 94°C (5 min), followed by 35 cycles of 94°C (30s), 48°C (45s) and 72°C (1 min) and a final extension phase at 72°C of 4 min. The amplicons were checked using 1% Agarose Gel and then PCR amplicons were purified by column purification to remove contaminants. The DNA was sequenced using Sanger's sequencing.

DNA barcoding

The obtained sequences were submitted to NCBI (National Centre for Biotechnology Information) to get accession ids. Later the details of each species along with the photos of the voucher specimen were submitted to BOLD (Barcode of Life Data System) to get a barcode.

Phylogenetic analysis

The Maximum likelihood method was used to construct the phylogeny and to draw the evolutionary relationship among the Coccinellid beetles. The pairwise distance was computed using the Maximum Composite Likelihood method in MEGA 11.0 software.

RESULTS AND DISCUSSION

The studies on genetic diversity among different Coccinellid beetles were carried out in the laboratory of the Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari. The molecular techniques employed in the present study include, i) DNA extraction, ii) PCR-based assays using COI gene for distinguishing different Coccinellid species iii) Utilization of COI sequences for studying the genetic variation and establishing evolutionary relationships among Coccinellid species.

DNA Barcoding

DNA was isolated from the samples and quality was evaluated on 1.0% agarose gel. After PCR amplification with primers LCO-HCO, gel electrophoresis was carried out on 1.0% agarose gel. The single distinct DNA band of approximately 650 bp length confirmed the amplification of COI gene. Both the forward and reverse sequences of 10 samples were sequenced by Sanger dideoxy method.

The forward and reverse sequences were aligned and contig sequences were generated. All the sequences were annotated in the NCBI-GenBank database using BLASTn tool with specimen data and relevant DNA sequences. All the sequences except H. implicata, J. assamensisand Novius sp., and S. castaneus had shown >95 percent similarity with the available sequences. A total of ten DNA sequences of ten Coccinellid species were deposited in the NCBI-GenBank (Table 1 and (Fig.1). Later, these sequences were submitted to DNA library (BOLD) and generated DNA barcodes for ten Coccinellid species from South Gujarat.

Table 1. List of GenBank accession IDs of Coccinellid species

S.No.	Species	GenBank Accession ID	Length of Sequences (bp)
1.	*Cheilomenessexmaculata*	ON564571	576
2.	*Coccinellatransversalis*	ON566029	309
3.	*Harmoniaoctomaculata*	ON568150	546
4.	*Harmonia implicate*	ON586690	548
5.	*Illeiscincta*	ON573329	533
6.	*Jauraviaassamensis*	ON689377	549
7.	*Novius*sp.	ON602031	492
8.	*Psylloborabisoctonotata*	ON593758	271
9.	*Scymnuscastaneus*	ON703377	541
10.	*Stethorus. pauperculus*	ON909191	555

Phylogenetic Analysis

The COI sequences of different Coccinellid species were aligned using ClustalW algorithm with the help of MEGA tools (version 11) with gap opening penalty 15, gap extensions penalty 6.66, transition weight 0.5 and delay divergent cutoff 30 percent [13 and 14].

In the present study, the phylogenetic tree was constructed with the highest log likelihood (-3892.11), using the Maximum likelihood

(ML) method and Kimura 2-parameter model [15] in order to draw the evolutionary relationship among the family Coccinellidae with ten species (10 genera) belonging to six tribes (Coccinellini, Epilachnini, Noviini, Scymnini, Stethorini, and Sticholotidini). The sequences of two species viz., Scelodontastrigicollis (KY672859) and Chaetocnemapulicaria (KJ207904) from the family Chrysomelidae retrieved from NCBI database were used as out-groups.

Table 2. Pairwise distance between different species of family Coccinellidae (Scelodontastrigicollis and Chaetocnemapulicaria as out-groups) based divergence in the COI region

	A	B	C	D	E	F	G	H	I	J	K	L
A												
B	0.174											
C	0.238	0.184										
D	0.228	0.198	0.218									
E	0.199	0.170	0.199	0.196								
F	0.233	0.238	0.292	0.257	0.238							
G	0.202	0.217	0.218	0.202	0.183	0.218						
H	0.258	0.219	0.244	0.233	0.184	0.259	0.178					
I	0.247	0.253	0.315	0.285	0.271	0.253	0.238	0.243				
J	0.247	0.270	0.297	0.258	0.233	0.243	0.212	0.259	0.248			
K	0.284	0.295	0.285	0.318	0.227	0.312	0.242	0.268	0.301	0.301		
L	0.278	0.284	0.273	0.295	0.284	0.263	0.227	0.278	0.296	0.259	0.274	

A-Cheilomenessexmaculata (ON564571), B-Coccinellatransversalis (ON566029),C- Harmoniaoctomaculata (ON568150), D-Illeiscincta (ON573329), E- Psylloborabisoctonotata (ON593758), F-Novius sp. (ON602031), G- Jauraviaassamensis (ON689377), H-Scymnuscastaneus (ON703377), I- Stethorus. pauperculus (ON909191), J- Henosepilachnaimplicata (ON586690), K-Scelodontastrigicollis (KY672859), L-Chaetocnemapulicaria (KJ207904)

The percentage of trees in which the associated taxa clustered together was shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter=0.3181)). The tree was drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). The analysis involved 12 nucleotide sequences with a total of 679 positions in the final dataset.

With regards to phylogenetic analysis, the species family Coccinellidae was demonstrated as a single clade for ML confirming the monophyly (Fig. 2). The monophyly of the family Coccinellidae was strongly supported by previous workers [16 and 17]. The species belonging to the tribe Coccinellini clustered together and formed a monophyletic clade. Earlier workers also reported the monophyly of tribe Coccinellini (Magroet al., 2010; Seagoet al., 2011). The present data do not support any pattern regarding the order of emergence and the origin of other tribes due to inadequate samples for the phylogenetic construction. However, each tribe formed a single clade. The tribes Epilachnini, Stethorini, and Sticholotidini were basal among the Coccinellidae. The tribe Noviini and Scymnini were closer and formed sister groups. In the context of branch length, Novius sp. was mostly evolutionary diverse species while C. transversalis was the least diverse.

The interspecific genetic distance among the Coccinellid species ranged between 0.170–0.315 (Table 2). The lowest genetic distance was found between C. transversalis and P. bisoctonotata (0.170) showing a close relation while the highest genetic distance was found between H. octomaculata and S. pauperculus (0.315) depicting a distant relation. The genetic distance among the tribe coccinellini ranged between 0.170–0.238 depicting a closer association which was also reflected in the phylogenetic tree (Fig. 2).

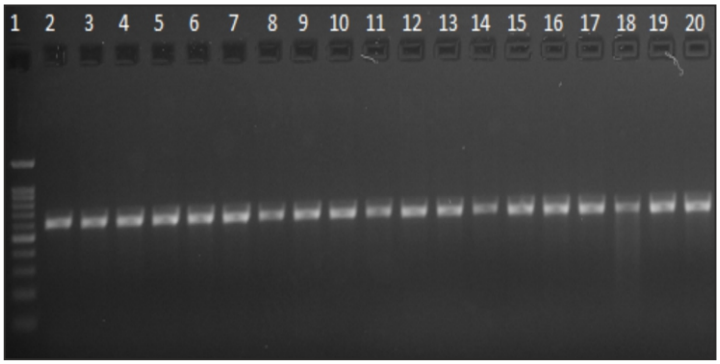

Fig. 1: Gel picture showing PCR amplifaction of COI gene 1) Ladder, 2) Cheilomenessexmaculata, 3) Coccinellatransversalis, 4) Harmoniaoctomaculata, 5) Henosepilachnaimplicata, 6) Illeiscincta, 7) Jauraviaassamensis, 8) Noviussp, 9) Psylloborabisoctonotata, 10) Scymnuscastaneus, 11) Stethoruspauperculus

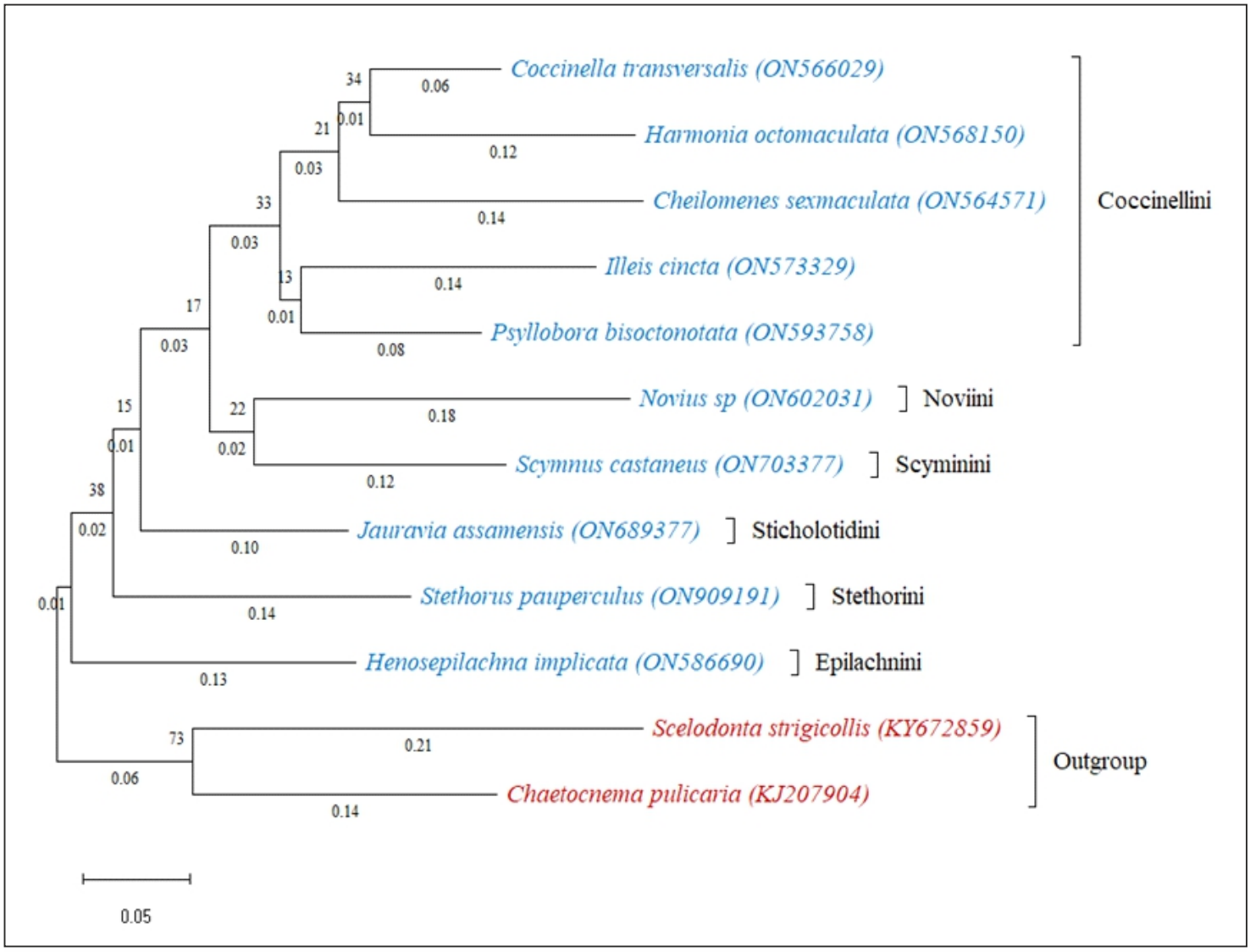

Fig. 2: Phylogenetic tree using Maximum Likelihood method for family Coccinellidae

ACKNOWLEDGEMENT

The authors are thankful to the Principal and Dean, N.M. College of Agriculture, Navsari as well as Director of Research and Dean, P.G. Studies, Navsari Agricultural University, Navsari for providing necessary facilities.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCE

1. Singh, P K and Nair, A (2012). Environmental sustainability of cropping patterns in Gujarat,Institute of Rural Management Anand, Anand, Gujarat, pp. 1–30.

2. Ślipiński, A and Tomaszewska, W (2010). Coccinellidae Latreille 1802. In: "Handbuch der Zoologie/Handbook of Zoology. Band/Volume IV. Arthropoda: InsectaTeilband/Part 38. Coleoptera, Beetles. Volume 2. Morphology and Systematics (Polyphagapartim)", (Leschen, R. A. B.; Beutel, R. G. and Lawrence J. F., Eds.), Berlin, Germany: W. DeGruyter. pp. 454–472.

3. Poorani, J (2008). Coccinellidae of the Indian Subcontinent. http://www.angelfire.com/bug2/j_poorani/index.html.

4. Poorani, J (2020). Coccinellidae of the Indian Subcontinent, In: "Indian Insects: Diversity and Science" (Ramani, S, Mohanraj, P, Yeshwant H. M. Eds.), CRC Press, Boca Raton, pp. 223–246.

5. Hebert, P D, Cywinska, A, Ball, S L and Dewaard, J R (2003). Biological identifications through DNA barcodes. Proc.R.Soc.B: Biol.Sci., 270 (1512): 313-321.

6. Hebert, P D, Stoeckle, M Y, Zemlak, T S and Francis, C M (2004). Identification of birds through DNA barcodes. Plos Biol., 2(10): e312.

7. BOLD, Barcode of Life Data System. http://www.barcodinglife.org.

8. Yan, L J, Liu, J, Möller, M, Zhang, L, Zhang, X M, Li, D Z and Gao, L M (2015). DNA barcoding of Rhododendron (Ericaceae), the largest Chinese plant genus in biodiversity hotspots of the Himalaya–Hengduan Mountains. Mol. Ecol. Resour., 15(4): 932–944.

9. Akram, S, Arshan, K M L and Abdul, J H (2017). DNA barcoding and phylogenetic analysis of five ascidians(Phlebobranchia) distributed in Gulf of Mannar, India. Mitochondrial DNA A, 29: 581–586.

10. Pentinsaari, M, Hebert, P D and Mutanen, M (2014). Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. PLoS One, 9 (9): e108651.

11. Oba, Y,Ôhira, H,Murase, Y, Moriyama, A and Kumazawa, Y (2015). DNA barcoding of Japanese click beetles (Coleoptera, Elateridae). PLoS One, 10(1): e0116612.

12. Raupach, M J,Hannig, K, Moriniere, J and Hendrich, L (2016). A DNA barcode library for ground beetles (Insecta, Coleoptera, Carabidae) of Germany: The genus BembidionLatreille, 1802 and allied taxa. ZooKeys, 592: 121–141.

13. Kobayashi, N, Tamura, K,Aotsuka, T and Katakura, H (1998). Molecular phylogeny of twelve Asian species of epilachnine ladybird beetles (Coleoptera, Coccinellidae) with notes on the direction of host shifts. Zool. Sci., 15 (1): 147–151.

14. Simon, S and Hadrys, H (2013). A comparative analysis of complete mitochondrial genomes among Hexapoda. Mol. Phylo. Evol.,69 (2): 393–403.

15. Kimura, M A (1980). Simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111–120.

16. Magro, A,Lecompte, E,Magne, F,Hemptinne, J L and Crouau-Roy, B (2010). Phylogeny of ladybirds (Coleoptera: Coccinellidae): are the subfamilies monophyletic?.Mol.Phylogenet.Evol.,54 (3): 833–848.

17. Seago, A E,Giorgi, J A, Li, J and Ślipiński, A (2011). Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Mol.Phylogenet.Evol.,60(1): 137–151.