https://doi.org/10.33307/entomon.v49i3.1254
Entomon 49(3): 343-354
Article No. ent.49305

Molecular characterization and identification of insect pollinators of *Valeriana jatamansi* Jones from Shimla Hills, Western Himalaya, India

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ABSTRACT: *Valeriana jatamansi* Jones a perennial medicinal herb belongs to the family Caprifoliaceae and highly pollinated by wild insect pollinators. In present investigation a total of 51 species of flower visitors were reported on *V. jatamansi* at altitude range of 1,927 to 2,850 m, in different localities of Shimla Hills, Western Himalaya. The collected insect pollinator species were taxonomically identified and 29 of them were molecularly characterized and identified using the cytochrome c oxidase subunit 1 (COI) sequence from mitochondrial DNA (mtDNA). BLAST analysis revealed 98 to 100 per cent similarity with the existing GenBank sequences. The average AT content was found to be significantly higher (69.8%) compared to the GC content (30.0%), indicating AT bias in all sequences. Phylogenetic analysis of 29 different species of insect 'pollinator of *V. jatamansi* revealed two clades, one shows phylogenetic relationship between 28 species which belongs to four orders Diptera, Hymenoptera, Hemiptera and Lepidoptera and the other shows the one species which belongs to order Coleoptera. This study contributes to increasing the number of published accounts of NCBI and helps to accurately distinguish the pollinator fauna of *Valeriana jatamansi*.

KEY WORDS: Medicinal herb, BLAST analysis, phylogenetic analysis, mtCOI sequences

INTRODUCTION

Pollination is a vital process that promotes biodiversity, agricultural productivity, wild plant reproduction, ecosystem health, and food security. It involves the interaction of plants and animals, and affects human well-being. They have a reciprocal link in which the survival of one of them benefits the other. Animals provide food, shelter, and space in exchange for being the primary pollinators of plants (Nepi *et al.,* 2018). Insects are one of the most significant types of pollinating organism. Insect pollination is vital to the reproduction and survival of several wild plants (Ollerton *et al*., 2011) including *Valeriana jatamansi*. In India, *V. jatamansi* belonging to the family Caprifoliaceae, is on the verge of extinction due to the over-exploitation of its roots/rhizomes from its natural habitat to meet the burgeoning industrial demand. It has become threatened and endangered plant species and is included in the endangered category by IUCN (Reveal and Chase,

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2011). This plant is commonly known as Indian valerian (English), Mushkbala (Kashmiri), Sughanthdawal, or Tagar (Sanskrit), is a native of Himalaya with extent distribution from Afghanistan to southwest China, India, Nepal, Bhutan, Myanmar and Burma. It is an important medicinal herb being exploited for its roots and rhizomes which contain valepotriates and is highly effective against leprosy epilepsy and hysteria (Maurya and Agnihotri, 2024), Parkinsons disease, Lewy body dementia (Bagchi and Hooper, 2011) and have anxiolytic properties (You *et al*., 2012). Cytotoxity of valepotriates has been reported for potential anti-tumor properties (Diapher and Hindwarch, 2004). It has a relatively high level of genetic diversity and therefore has greater environmental adaptability. The adaptability is also demonstrated by the distribution of this species, which extends between a wide altitudinal range of 1000 to 3000m elevation, and is generally preferred to grow on hill slopes, moist places, damp woods, ditches, and along streams (Rather *et al*., 2012; Jugran *et al*., 2013). The flowering and fruiting time for the species is March–June (Jugran *et al*., 2013).

The identification of insects via the conventional approach is fairly challenging because of the morphological alterations due to seasonal and regional variances. Organisms adapt themselves physiologically and morphologically to flourish in difficult environments. According to Jalali *et al*. (2015), genetic studies are subsequently essential to the identification and phylogenetic study of organisms at the species level. While morphological data are generally time-consuming and require expertise, DNA barcoding methods offer a standardized and practical means of species identification for insects. Molecular identification and showing phylogenetic connections employing COX 1 of the mitochondrial area as species identification markers are considered effective since mitochondrial DNA changes fast as compared to nuclear DNA. Present-day mitochondrial DNA (mtDNA) serves as among the most widely utilized molecular markers. Since mtDNA is maternally inherited, it changes quite quickly, and most of the nucleotide modifications occur at neutral sites. With relation to this genetic marker, the intra and inter-phylogenetic links have been studied employing the sequencing data acquired from the COX 1 marker gene amplification (Jalali *et al.,* 2015).

The purpose of this study was to explore the molecular characterization and identification of insect pollinators of *Valeriana jatamansi* using mitochondrial DNA sequences of the COI barcode dataset. The main goals of this study were to examine the utility and effectiveness of the mtDNA (COI) marker for identifying insect species, compared to traditional taxonomic identification methods. This research aims to demonstrate the practical use of the mtDNA marker for species identification in insect pollinators of *V. jatamansi*, given the medicinal significance and endangered status of the plant. Precisely identifying the pollinator fauna of *V. jatamansi* could help protect wild pollinators and improve plant yields.

MATERIALS AND METHODS

The present investigation was carried out on insect pollinators of *V. jatamansi* in eight localities of Shimla Hills, Western Himalayas, viz., Tara Devi (1,927m; 31°04’06"N: 77°07’24"E), Dhalli (2,155m; 31°06'39"N: 77°11'53"E), Chaura Maidan (2,100m; 31°06'34"N: 77°08'34"E), Fagu (2,576m; 31°05'22"N: 77°18'05"E), Matiyana (2,419m; 31°12'36"N: 77°24'11"E), Kufri (2,609m; 31°06'01"N: 77°16'02"E), Kharapather (2,703m; 31°07'13"N: 77°37'37"E) and Kupper (2,850m; 31°06'00"N: 77°36'13"E). Collection of insect pollinators were done during the flowering season i.e., from March to May, in the years 2018 to 2021 and during this period collected specimens were preserved at -80^{0}C in deep freezer for molecular analysis. During study the relative abundance of different insect pollinators on *V. jatamansi* was determined in terms of their visit per 500 flowers/10 minutes. The observation was recorded during 0900–1000, 1200–1300, and 1500–1600 hours of a day, and the average count at these hours gives an abundance of insect pollinators for that particular day (Mattu and Kumar, 2016). In order to ascertain whether pollen grains were present, each specimen was put into a distinct vial, frozen and then rinsed in 0.1ml of 70 per cent ethanol. Pollen was cleaned onto a glass slide and then stained with carbol fuchsin. Pollen grains were

identified by comparing them with pollen removed from flowers harvested from the plant. All the preserved specimens of insect pollinators from different altitudinal population of Shimla Hills, Himachal Pradesh were identified based on the diagnostic morphological features, wing venation and genital characteristics. Identification was done with the help of earlier records of the Entomology and Biodiversity Laboratory of the Department of Biosciences, Himachal Pradesh University, High Altitude Regional Centre, Zoological Survey of India, Solan, Zoological Survey of India, Kolkata and also by consulting literature.

Genetic analysis: DNA was extracted from the legs or thorax of the insect specimen by using DNeasy blood and tissue Qiagen kit method and extracted DNA was preserved in the -20^{0}C for further use. Integrity and presence of DNA was checked on 1.2% agarose gel electrophoresis and DNA was visualized under UV transilluminator as bright bands. Target DNA from mitochondrial gene, i.e., Cytochrome Oxidase subunit I was amplified by using a pair of forward primers LCO1490 5'-GGT-CAA-CAA-ATC-ATA-AAG-ATA-TTG-G-3' and reverse primer HCO2198 5'- TAA-ACT-TCA-GGG-TGACCA-AAA-AAT-CA-3' (Folmer *et al.,* 1994). Polymerase chain reaction (PCR) was performed in 96-well plates with 20 ml reaction volume (1mL DNA template; 1 mL forward primer; 1 mL reverse primer; 5 mL distilled water; 12 mL emerald PCR master mix) in a C1000 thermal cycler. The amplified product was analyzed on a 1.2% agarose gel electrophoresis and checked under UV light and documented. The amplified DNA fragments were extracted from agarose gels and purified using DNA/RNA purification Qiagen kit method. The primers used were the same primers used in PCR amplification and sequencing was done in Big dye terminator version 3.1" cycle sequencing kit with sequencing machine (ABI 3500xL Genetic analyzer). After completion of sequencing, the results were analyzed by using MEGA 11 software (Kumar *et al.,* 2018). Analyses were performed on 1000 bootstrapped data sets generated by the program (Felsenstein, 1985).

Sequences and Phylogenetic analysis: All fasta format sequences obtained by Sanger sequencing were used for BLAST search in NCBI. All the sequences were edited and aligned by using bio edit (7.2.5 software) sequence alignment editor software. All the gaps and mismatched data were removed and sequences were submitted in the NCBI GenBank for accession number. The pairwise analysis of 29 sequences of different insect pollinators species obtained by using the Neighbor-joining bootstrap method and the Kimura-2 parameter in MEGA11 software. The number of base substitutions per site was analyzed between all sequences. Codon positions included were 1st+2nd+3rd+non-coding. All positions containing gaps and missing data were eliminated from the dataset. All sequences A, T, G, C, AT and GC content were obtained using MEGA 11 software. The AT per cent at three codon positions was calculated using the same program. Sequences were aligned using the MEGA 11 software package.

RESULTS AND DISCUSSION

Fifty-one species of insect pollinators belonging to 5 orders and 12 families of the class Insecta were reported on *Valeriana jatamansi* during the study period. Thirty three species belonged to order Diptera, nine to Lepidoptera, four to Hymenoptera, three to Coleoptera, and two to Hemiptera (Table 1). Diptera was most dominant order represented by 33 (64.71%) insect pollinator species belonging to four families, viz., Syrphidae (26), Tachinidae (4), calliphoridae (2) and bombyliidae (1) with syrphidae (51%) being the most dominant family. Relative abundance study shows that dipterans were the most abundant insect pollinators in all eight localities i.e. Tara Devi (80.14%), Dhalli (84.97%), Chaura Maidan (87.59%), Fagu (91.10%), Matiyana (63.2%), Kufri (92.62%), Kharapather (82.73%), and Kupper (82.91%), followed by hymenopterans, lepidopterans, hemipterans and coleopterans. Among all the insect pollinators, *Sphaerophoria indiana* was the most abundant insect visitor on *V. jatamansi* flowers in all localities [Tara Devi (32.98%), Dhalli (23.39%), Chaura Maidan (19.38%), Fagu (19.70%), Matiyana (13.51%), Kufri (9.06%), Kharapather (11.05%) and Kupper

Table 1. Systematic list of insect pollinators of *Valeriana jatamansi* Jones from different localities of Shimla Hills, western Himalaya

Order	Family	Fauna/ Insect species
Diptera	Syrphidae	*Eristalis tenax* (Linnaeus, 1758)
		Eristalis cerealis Fabricius,1805
		Eristalis himalayensis Brunetti, 1908
		Eristalinus arvorum (Fabricius 1787)
		Eristalinus megacephalus (Rossi, 1794)
		Eristalinus paria (Bigot, 1880)
		Episyrphus balteatus (De Geer, 1776)
		Syrphus vitripennis Meigen, 1822
		Syrphus torvus (Osten Sacken, 1875)
		Melanostoma mellinum (Linnaeus, 1758)
		Melanostoma scalare (Fabricius, 1794)
		Melanostoma orientale (Wiedermann, 1824)
		Sphaerophoria indiana Bigot, 1884
		Syritta pipiens (Linnaeus, 1758)
		Eupeodes luniger (Meigen, 1822)
		Eupeodes latifasciatus (Macquart, 1829)
		Parasyrphus lineolus (Zetterstedt, 1843)
		Chrysotoxum baphyrus Walker, 1849
		Scaeva pyrastri (Linnaeus, 1758)
		Meliscaevia cinctella (Zetterstedt, 1843)
		Rhingia laticincta Brunetti, 1907
		Eumerus aurifrons (Wiedermann, 1824)
		Dasysyrphus lenensis Bagatshanova, 1980
		Platycheirus nigrofemoratus (Kanervo, 1934)
		Platycheirus albimanus (Fabricius, 1781)
		Brachyopa notata Osten Sacken, 1875
	Tachinidae	*Gymnosoma sylvaticum* (Zimin, 1966)
		Nowickia marklini (Zetterstedt, 1838)
		Tachina fera (Linnaeus, 1761)
		Estheria petiolata (Bonsdorff, 1866)
	Calliphoridae	*Calliphora vomitoria* (Linnaeus, 1758)
		Lucilia papuensis Macquart, 1843
	Bombylidae	*Bombylius major* Linnaeus, 1758

Hymenoptera	Apidae	*Apis cerana* Fabricius, 1793
		Apis mellifera Linnaeus, 1758
	Halictidae	*Lasioglossum minutissimum* (Kirby, 1802)
	Megachilidae	*Megachilidae* sp.
Coleoptera	Coccinellidae	*Coccinella septempunctata* (Linnaeus, 1758)
		Hippodamia variegate (Goeze, 1777)
		Oenopia sexareata (Mulsant, 1853)
Hemiptera	Miridae	*Pinalitus rubricatus* (Fallen, 1807)
		Orthops scutellatus Uhler, 1877
Lepidoptera	Pieridae	*Pieris canidia* (Sparrman, 1768)
		Colias electo fieldi Ménétriés, 1855
		Eurema laeta Boisduval, 1836
		Eurema hecabe (Linnaeus, 1758)
	Nymphalidae	*Neptis mahendra* Moore, 1872
	Lycaenidae	*Heliophorus sena* (Kollar, 1844)
		Heliophorus androcles (Westwood, 1851)
		Celastrina lavendularis (Moore, 1877)
		Celastrina huegeli (Moore, 1882)

(16.00%)].

Jugran *et al*. (2013) conducted a similar experiment on *V. jatamansi*, discovered that 13 species of dipterans were the most dominant visitors to its blooms. Hymenopterans, lepidopterans, and other minority groups were also flower visitors. The present outcome is supported by the recent finding of Katoch and Thakur (2022); they detected 29 pollinator species from the orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera, on two plants *Bergenia ciliata* (Haw.) Sternb. and *Vinca major* in the Western Himalayas. Kumari *et al*. (2021) studied how a reduction in flower size influenced the pollination and reproduction of *V. wallichii*, an endangered medicinal plant and observed that entomophily was the method of pollination.

All 51 species are taxonomically characterized during study which belongs to five orders i.e. Diptera, Hymenoptera, Lepidoptera, Coleoptera and Hemiptera. Dipterans are characterized by having one pair of forewings and hindwings are modified into halteres. Hymenopterans are small and medium sized insects with two pairs of wings and a narrow waist that set off abdomen from the thorax. Lepidopterans forewings and hind wings have large surface area, body and wings are mostly covered with tiny colored scales. Coleopterans are distinguished on the basis of sclerotized forewings called elytra and a pair of membranous hind wings. Hemipteran wings are partly membranous and partly harden known as hemelytra. Dipterans showed similar wing venation patterns but showed differences in vein R_{4+5} in different species. Species of family Syrphidae differentiated on the bases of their wings. They have a unique vein vena spuria which is absent in other dipterans. In *Eristalis tenax* spurs were present in the bm-cu region. Tachinid are minute to very large flies with extremely diverse appearance, often extremely bristled. Nearly all adult tachinids have a distinct bend in M vein and absence of vena spuria. All dipterans have a large

Table 2. Sequencing with accession number and BLASTN analysis on 29 insect pollinators on *Valeriana jatamansi* Jones

Order	Family	Samples with accession no.	BLASTN results	Query cover (%)	Identical (%)
Diptera	Syrphidae	*Eristalis tenax* OK598005	MN967352	100	100
		Eristalis cerealis OL454830	OL440713	100	100
		Eristalis himalayensis OL442159	MW307783	100	100
		Eristalis arbustorum OP393912	MN868881	100	100
		Episyrphus balteatus OP847796	OL405702	100	100
		Melanostoma scalare OK639012	MN481519	100	98.85
		Syrphus torvus OP363961	KT959674	100	99.84
		Syrphus vitripennis OL305851	KR657522	100	100
		Eupeodes luniger OP363960	KY834510	100	100
		Syritta pipienes OL454816	MN868864	100	100
		Parasyrphus lineolus OP380744	MZ609220	100	100
		Scaeva pyrastri OP788181	HQ944919	100	100
		Dasysyrphus lenensis OP363531	KM930046	98	100
		Platycheirus nigrofemoratus OP363759	HQ577938	99	100
		Platycheirus albimanus OP363294	NC056282	100	99.05
	Tachinidae	*Gymnosoma sylvaticum* OP379750	MT048383	100	100
		Nowickia marklini OP393894	HM861393	100	100
		Tachina fera OL445006	LR999969	100	100
Hymenoptera	Apidae	*Apis mellifera* OP847793	MW428265	100	99.56
	Halictidae	*Lasioglossum minutissimum* OP393898	KT164664	100	100
Coleoptera	Coccinellidae	*Coccinella septempunctata* OL539601	XM898241	100	100
Hemiptera	Miridae	*Pinalitus rubricatus* OP393894	KM022967	100	100
		Orthops scutellatus OP380608	KR032953	100	99.61
Lepidoptera	Pieridae	*Pieris canidia* OP788182	MT935585	100	100
		Eurema laeta OK639008	GU372560	100	99.55
		Eurema hecabe OP393888	OL343184	100	99.51
	Nymphalidae	*Neptis mahendra* OP788197	OK342272	100	100
	Lycaenidae	*Heliophorus sena* OK639007	KC755862	99	100
		Heliophorus androcles OP363632	KT236373	100	99.81

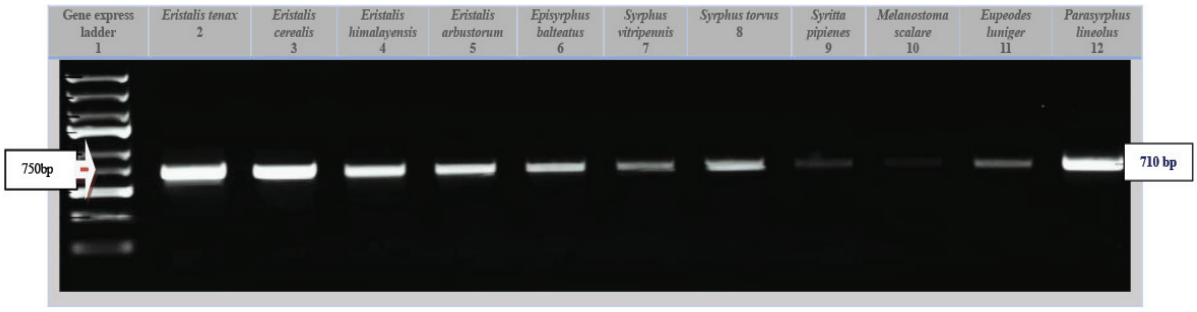

Fig. 1 Analysis of amplified PCR product in 1.2% agarose; Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5, 6, 7, 8, 9, 10 11, 12: 710 bp size mtCOI gene

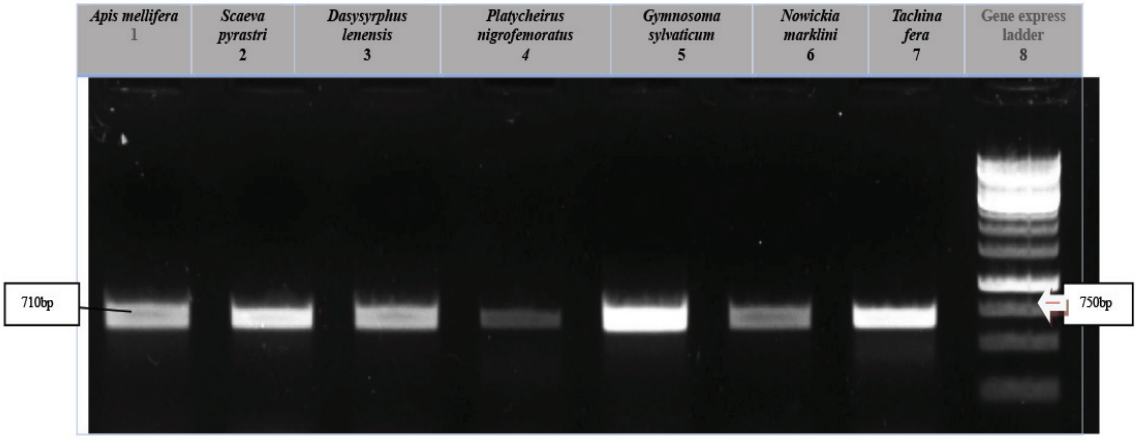

Fig. 2 Analysis of amplified PCR product in 1.2% agarose; Lane 8: Gene ruler express DNA ladder, Lane 1,2, 3, 4, 5, 6, 7: 710 bp size mtCOI gene

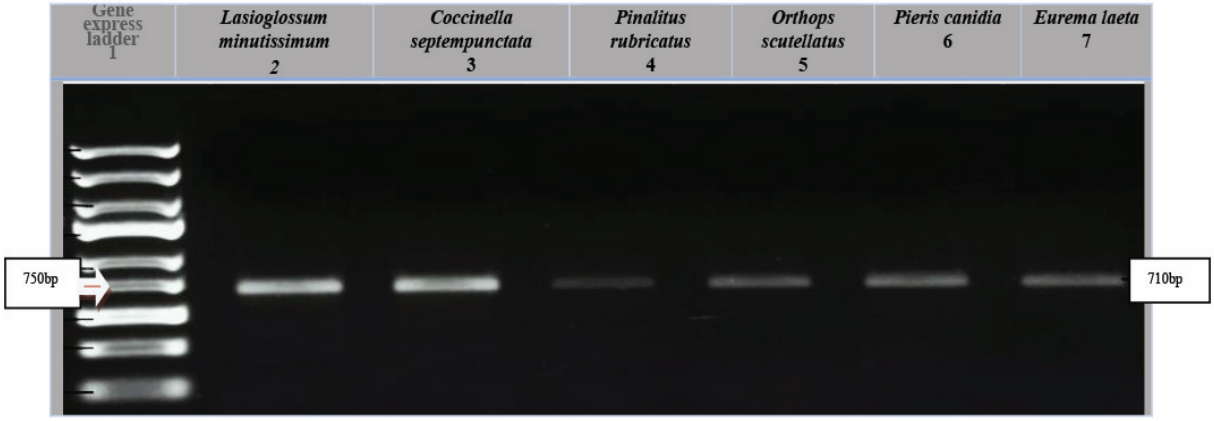

Fig. 3 Analysis of amplified PCR product in 1.2% agarose; Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5, 6, 7: 710 bp size mtCOI gene

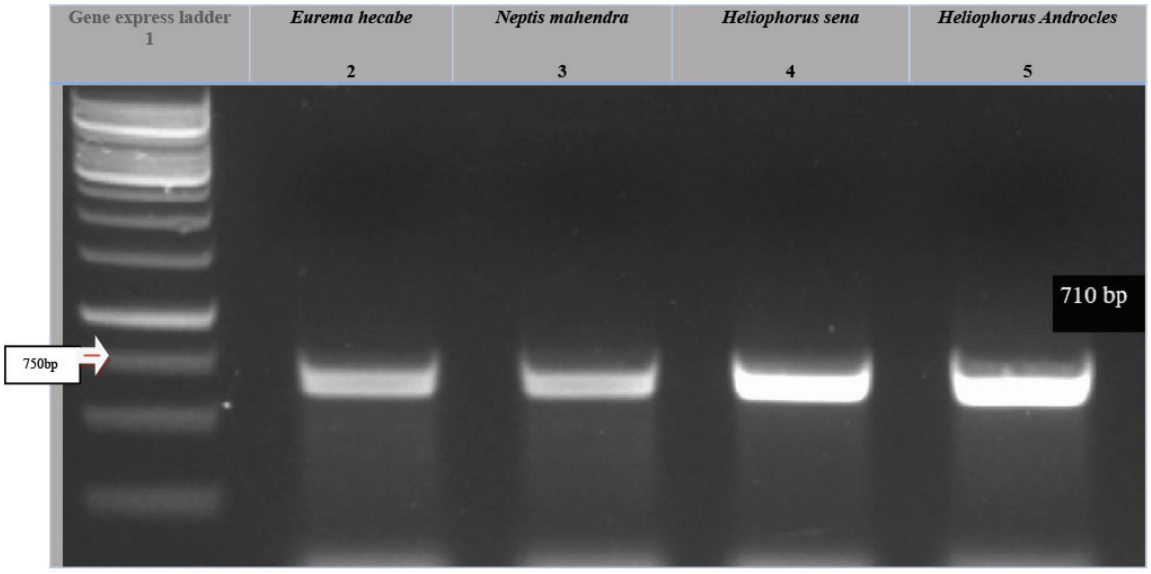

Fig. 4 Analysis of amplified PCR product in 1.2% agarose; Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5: 710 bp size mtCOI gene

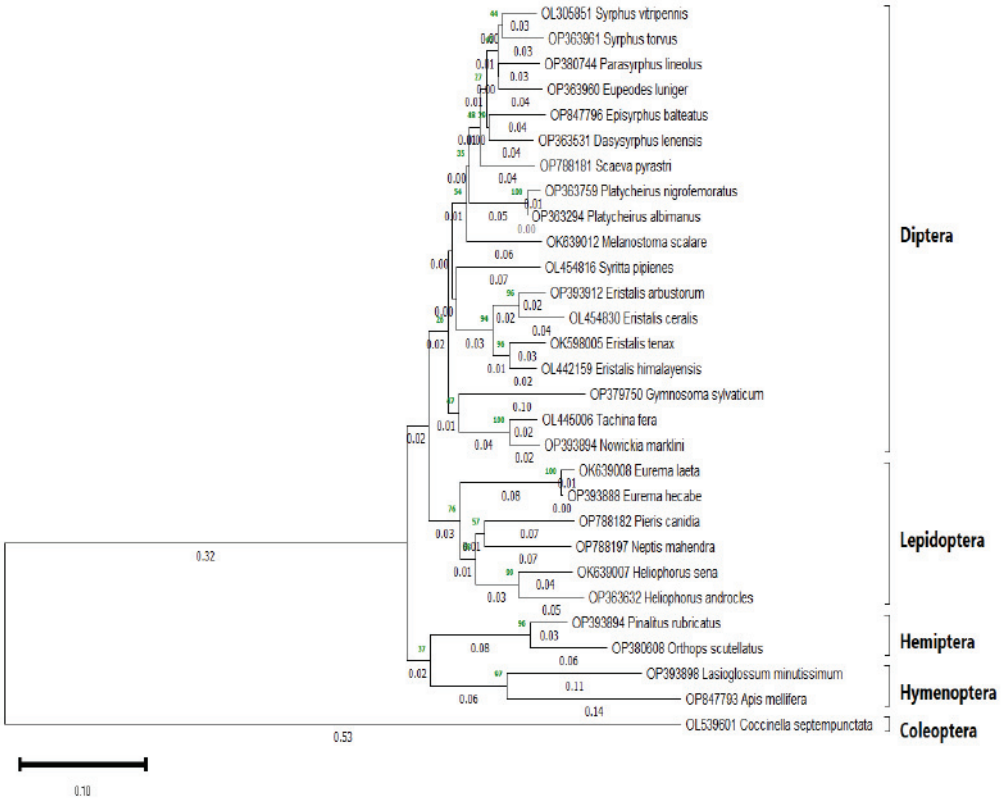

Fig. 5 Phylogenetic tree for 29 insect pollinators of insect pollinator species showing genetic relationship derived from COI sequences by using Neighbor-Joining (NJ) method of MEGA 11 Software

genital tergite located apico-ventrally, partially surrounded by a pair of lobular structures called cerci pair of hairy surstyli, and a large, typically thick superior lobe. While collecting the hymenopterans, most of specimens were workers and it has been observed that genitalia are modified into sting apparatus. Sting apparatus in *Apis mellifera, A. cerana*, *Bombus ardens,* and *Lasioglossum minutissimum* contains a barbed lancet.

When it comes to dipterans, the present findings are similar to the research conducted by Miranda *et al.* (2013), who created an interactive photographic key that covers all genera of Syrphidae in the Nearctic Region. Miranda and Moran (2017) also studied the female abdomen and genitalia of Syrphidae and examined the presence of sclerotized areas on the intersegmental membrane that appears in Rhingiini. Sengupta *et al.* (2018) discovered four new records from Himachal Pradesh's cold, dry zones when they investigated the taxonomic account of hover flies. They described eighteen species of hoverflies from fourteen genera and two subfamilies found in the Himalayan cold and dry zone. The present study is identical with the study of Belyaev and Farisenkova (2019) on the allometry of wing shape and wing venation in dipterans and they observed that the arrangement of veins varied significantly between different families. Rana and Thakur (2019) conducted a comprehensive experiment focusing on the biodiversity of butterflies in the Dharampur region, located in the Mandi district of Himachal Pradesh. Their study involved the collection and analysis of 33 distinct butterfly species, which were classified into 25 genera, spread across six families and two superfamilies.

Rego *et al.* (2022) represents a pictorial key for the identification of 26 species of hoverflies. Pathania and colleagues (2022) conducted a comprehensive study on the physical attributes of the queen, workers, and drones of the *A. mellifera* species in the Kangra district of Himachal Pradesh. They meticulously measured and analyzed 10 distinct features of these *A. mellifera*.

Among all the insect pollinators attempted to sequence during molecular analysis, only 29 insect species of pollinators were able to successfully sequence using Sanger sequencing with mtDNA markers. These markers included the cytochrome oxidase subunit sequence I (COI) (Table 4). All the sequenced genes showed 98 to 100% similarity with the existing GenBank sequences in the BLAST analysis (Table 2). The CO1 region in almost all the samples was in the range of 710bp (Fig. 1– 4). All sequences were submitted in the NCBI GenBank for accession number.

Nucleotide composition of COI gene sequences: The nucleotide content (A,T,G,C) and the total C+G and A+T at first, second and third codon position of all the samples revealed that the average AT content was significantly higher by 69.8 per cent than the GC content of 30per cent. Sequences were deeply AT-biased due to 3rd codon position, which is expected in insect mtDNA. The high numbers of polymorphic sites were uniformly distributed throughout the third codon position in the COI gene. For all codon locations in this area, the A+T bias was very strong. In *V. jatamansi* there were a total of 684 positions in the final dataset. Evolutionary analyses were conducted in MEGA11software. Average genetic distances between the diverse groups of insects used in this study showed higher values at the third codon position (93.2%), indicating that further research of the third codon location for insects could disclose possible evolutionary information between these closely related groups of insects (Table 3).

Phylogenetic analysis: Phylogenetic tree for DNA sequences of 29 species of insect pollinators collected on *Valeriana jatamansi* was constructed by the Neighbor-joining method. The tree was divided into two clades, one shows the phylogenetic relationship between Diptera, Hymenoptera, Hemiptera and Lepidoptera and the other clade shows the phylogenetic relationship between Coleoptera. Out of 29 species first clade consists of 28 species, 18 belongs to Diptera, two to Hemiptera, six to Lepidoptera and two to Hymenoptera and second clade constitute only one species which belonged to order Coleoptera. Phylogenetic analysis of Diptera formed two

distinct groups which included family Syrphidae in one group and Tachinidae in another group. In Lepidoptera, two groups were formed included species belonging to families Pieridae, Nymphalidae and Lycaenidae. Hemipterans formed one distinct group of two species from family Miridae and Hymenoptera showed one species each from family Apidae and Halictidae. The Phylogenetic tree had a total branch length of 2.62 base substitutions per site. The study concluded that dipterans, hymenopterans, hemipterans and lepidopterans arise from common ancestor as compare to coleopterans (Fig. 5).

Molecular characterization of 29 insect pollinators species collected on *Valeriana jatamansi* Jones have been performed by mtCOI sequences. The average AT content was 69.8 per cent, while the GC content was 30 per cent. The sequences exhibited a strong bias towards AT in the third codon position, which is in line with expectations for insect mtDNA. Average genetic distances across the numerous groups of insects employed in this research revealed greater values at the third codon position, suggesting that future examination of the third codon site for insects could offer probable evolutionary evidence among this closely related group of species. All the species were phylogenetically very closely related to each other. Above results are in accordance with the earlier findings of Jalali *et al.* (2015) identified agriculturally important insects using DNA barcoding. They found that AT content was significantly higher than GC content, and phylogenetic analysis showed two clades, one consisting of hymenopteran insects and the other consisting of other orders.

Mitochondrial DNA analysis of COI sequences is a standardized, accurate, efficient, and time-saving method to illustrate and identify insect pollinator species, as compared to conventional taxonomic identification which is time consuming and require expertise. Molecular biologists around the world have been using mitochondrial cytochrome oxidase subunit I (COI) to identify insect species. Kumar *et al.* (2012) identified medically important insects in India through DNA barcoding, identifying seven morphologically identified species of *Phlebotomus* and *Sergentomyia*. Prabhakar *et al.* (2013) analyzed the population genetic structure of the pumpkin fruit fly, *Bactrocera tau*, using mitochondrial cytochrome oxidase I (mtCOI) gene sequences. Karthika *et al.* (2016) assessed the

Table 3. AT % at the First, Second and Third Codon of different insect species of *Valeriana jatamansi* Jones

Samples	1st	2nd	3rd	Total	
	AT	AT	AT	C+G	A+T
Eristalis tenax	56.5	56.2	93.9	30.9	68.8
Eristalis cerealis	56.9	54.2	92.9	31.8	68.0
Eristalis himalayensis	56.9	55.7	95.8	30.3	69.5
Eristalis arbustorum	56.9	54.7	97.2	30.2	69.7
Episyrphus balteatus	58.0	54	98.9	29.5	70.3
Melanostoma scalare	56.8	53.6	96.7	30.8	69.0
Syrphus vitripennis	57.3	54.1	99.0	29.7	68.8
Syrphus torvus	57.1	53.8	95.6	31.0	70.1
Eupeodes luniger	57.3	53.3	97.5	30.5	69.3
Syritta pipienes	59.9	52.1	92.1	31.8	68.0
Parasyrphus lineolus	57.0	53.1	96.5	31.0	68.8
Scaeva pyrastri	57.5	53.0	96.6	30.7	69.1
Dasysyrphus lenensis	57.6	52.6	97.2	30.7	69.1
Platycheirus nigrofemoratus	56.7	54.6	93.8	31.5	68.4
Platycheirus albimanus	56.9	52.6	99.0	30.3	69.5
Gymnosoma sylvaticum	58.0	58.3	91.6	30.6	69.2
Nowickia marklini	57.0	53.2	96.1	31.0	68.8
Tachina fera	57.9	54.3	96.0	30.4	69.3
Apis mellifera	61.6	65.8	95.9	25.5	74.5
Lasioglossum minutissimum	60.7	66.2	90.6	27.3	72.6
Coccinella septempunctata	58.0	62.9	85.2	31.0	68.8
Pinalitus rubricatus	59.3	59.7	88.1	30.7	69.1
Orthops scutellatus	57.8	57.8	82.3	33.8	66.0
Pieris canidia	58.1	58.3	90.5	30.9	69.0
Eurema laeta	58.2	57.5	88.1	31.7	68.0
Eurema hecabe	56.1	57.6	86.7	33.1	66.8
Neptis Mahendra	57.9	57.9	91.0	30.8	68.9
Heliophorus sena	59.5	61.3	91.6	29.0	70.8
Heliophorus androcles	59.1	62.8	89.2	29.4	70.4
Average	57.8	56.5	93.2	30.5	69.2

DNA barcoding and evolutionary ancestry of 15 insect pests of agricultural crops in South India, finding high divergence among insect pests. Khullar *et al*. (2016) studied six forensically relevant blowfly species from India, using mitochondrial cytochrome oxidase subunit I (COI) DNA as an identifying marker. Kaur and Singh (2020) described the evolutionary significance of the pentatomid insect using mitochondrial COI gene sequences, finding an A+T concentration of 65.8 per cent and a R value of 1.39. Molecular Characterization of Pollinators in cotton ecosystem done by Bajaj *et al*. (2023) revealed that specimens collected in cotton belong to the Hymenoptera and Diptera orders.

ACKNOWLEDGEMENTS

Authors are very grateful to Director and scientists (Dr/late Sundaresha Sidappa, Dr. Kailash Naga) and staff members (Mr. Chandermohan Singh Bist) of ICAR-CPRI, Shimla for their cooperation and providing lab facility during the molecular work. Big thanks to ICMR for guidance and provide financial assistance through ICMR-JRF fellowship.

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(Received February 03, 2024; revised ms accepted July 05, 2024; published September 30, 2024)