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Genetic Diversity of *Eristalis Tenax* (Linnaeus, 1758) as Insect Pollinator of *Prunus Persica* (L.) Stokes Flowers Based on MtcoI Gene

Poonam Dhiman and Mahender Singh Thakur*

Abstract

Eristalis tenax is an important insect pollinator of *Prunus persica* plant and samples were collected from seven localities of Himachal Pradesh i.e. Bathra (503m), Jaladi (508m), Hamirpur (786m), Rajgarh (1555m), Naldera (1887m), Summer Hill (2100m) and Mashobra (2146m). Phylogenetic relationship and multiple sequence alignment of all the sampled sequences of *Eristalis tenax* were analyzed by using mtCOI gene. Nucleotide composition analysis showed that percentage of A+T (69.03%) was higher than the percentage of C+G (30.97%) which showed that all the sequences were AT biased. Multiple sequence alignment showed three variable sites in the sequences of *Eristalis tenax* and the comparable values of transitions and transversions in the current study suggest the possible occurrence of genetic divergence over evolutionary time scales in *Eristalis tenax* of Himachal Pradesh.

Keywords: *Eristalis tenax*; *Prunus persica*; mtCOI; Nucleotide composition; Multiple sequence alignment

Introduction

Pollinators are an essential part of the world's biodiversity because of their crucial ecological services to plants and crops. Pollinators enhance the genetic diversity in plants by cross pollination, that's why they considered essential for the survival and maintenance of diversity of plants [1]. But at present time, pollinator populations have been declining due to habitat degradation, climate change, pollution and over-exploitation. Due to these reasons, many insect pollinators population have been reduced to small isolated fragmented groups and are under high risk of extinction [2]. The fundamental objective to study the genetic diversity is to use genetics knowledge to reduce the risk of pollinators extinction. Genetic diversity helps a species to adjust according to changing environment and maintain higher level of biodiversity at population and species level [3].

Material and Methods

In the present study, samples of *Eristalis tenax* were collected from seven localities of Himachal Pradesh from *Prunus persica* flowers (Table 1). DNA was extracted from the thorax or upper abdominal region of the collected insect specimens by using DNeasy blood and tissue Qiagen Kit method by following standardized protocol of the manufacturers. Extracted DNA was preserved in the -20°C for further use. Target DNA from mitochondrial gene, i.e. Cytochrome Oxidase subunit I was amplified using a pair of forward primers LCO1490 5'- GGTCAACAAATCATAAAGATATTGG-3' and reverse primer HCO2198 5'- TAAACTTCAGGGTGACCAAAAAATCA-3'

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[4]. Total volume of PCR reaction was 20 µl and reaction run in PCR as per standardized protocol.

The amplified PCR 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 gel and purified using DNA/RNA purification Qiagen Kit. 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 a sequencing machine-ABI 3500xL Genetic analyzer. After completion of sequencing, all fasta format sequences obtained by Sanger sequencing was used for BLAST search to check the sequence homology at NCBI. All the sequences were edited and aligned using bioedit sequence alignment editor software. The edited sequences were submitted in the gene bank for accession Number (Table 1). The nucleotide content (A, T, G, C) of all the samples and the total C+G and A+T at first, second and third codon position were calculated using MEGA X software. DNADIST with the Kimura two parameter distance options were used to estimate divergence between sequences with a transition/ transversion ratio in the MEGA X software. Evolutionary analysis of obtained 7 sequences of sampled *Eristalis tenax* were conducted using Neighbor-Joining method and Kimura-2 parameter in MEGA X. Sequences were aligned by using the MEGA X software [5]. Analyses were performed on 1000 bootstrapped data sets generated by the program [6].

Results and Discussion

In the present study, DNA of *Eristalis tenax* collected from different altitudes of Himachal Pradesh was extracted and amplified using forward and reverse primers (Figure 1). The amplified products were sequenced by using Sanger sequencing and obtained fasta files were used to BLAST search in NCBI. The fasta format sequences were edited and mismatches were removed by using Bioedit sequence alignment editor software. The edited sequences were submitted in Genbank for accession number. Each sequence was accessed with accession number (Table 1). Multiple sequence alignment of seven sequences were performed by CLUSTAL Omega (1.2.4), which revealed three variable sites in COI sequences of *Eristalis tenax* (Figure 2).

Nucleotide content analysis of COI gene

The estimated transition/transversion bias (R) is 2.56 and the sites showing transition (75.42%) was higher than the sites showing transversion (24.6%) (Table 2). The total nucleotide content was 31.78% (A), 37.43% (T/U), 15.70% (C) and 15.01% (G). DNADIST with the Kimura two parameter distance option was used to estimate divergence between sequences with transition/ transversion ratio in the MEGA X software.

Base composition at each codon positions

The percentage of A+T (69.03%) was higher than the percentage of C+G (30.97%) which showed that all the sequences were AT biased (Table 3). Nucleotide content of all the samples and total A+T and C+G were calculated by MEGA X software. Transition/transversion (Ts/Tv) ratio helpful in determining the degree and direction of natural selection. Study showed that overall transition/transversion bias (R) was 2.56 which indicated the positive or Darwinian selection in *Eristalis tenax* sequences of Himachal Pradesh. The comparable values of transitions and transversions in the current study suggest the possible occurrence of genetic divergence over evolutionary time scales.

Phylogenetic analysis of mitochondrial *Eristalis tenax* COI sequences

Phylogenetic analysis of mitochondrial COI sequences was studied by constructing phylogenetic tree by Neighbor-Joining (NJ) method to study similarity and differences among different sequences (Figure 3). The phylogenetic relationship between seven COI sequences showed that the sequences of Mashobra and Naldera shared homology with each other and were also similar to the sequence of the Bathra. Summer Hill sequence found similar with Hamirpur. The sequence of Jaladi and Rajgarh were distinct from the other sequences. Distance matrix clearly signifies the very less difference among the sampled sequences shows that there is very less genetic diversity between them (Figure 3).

Table 1: Localities of sample collection of *Eristalis tenax* with geographical location and the Genbank accession numbers of COI gene.

S. No.	Taxon	Sample Location	Geographical Location			Genbank Accession No.
		Locality	Longitude	Latitude	Altitude	COI
1	*Eristalis tenax*	Bathra	76°-21′46	31°-88′18	503 m	OK444106
2	*Eristalis tenax*	Jaladi	76°-34′44	31°-77′85	508 m	OK465102
3	*Eristalis tenax*	Hamirpur	76°-52′13	31°-68′62	786 m	OK559908
4	*Eristalis tenax*	Rajgarh	77°-29′94	30°-85′00	1555 m	OL589625
5	*Eristalis tenax*	Naldera	77°-18′69	31°-18′39	1887 m	OQ359958
6	*Eristalis tenax*	Summer Hill	77°-13′99	31°-11′46	2100 m	OK655835
7	*Eristalis tenax*	Mashobra	77°-22′83	31°-12′96	2146 m	OL589638

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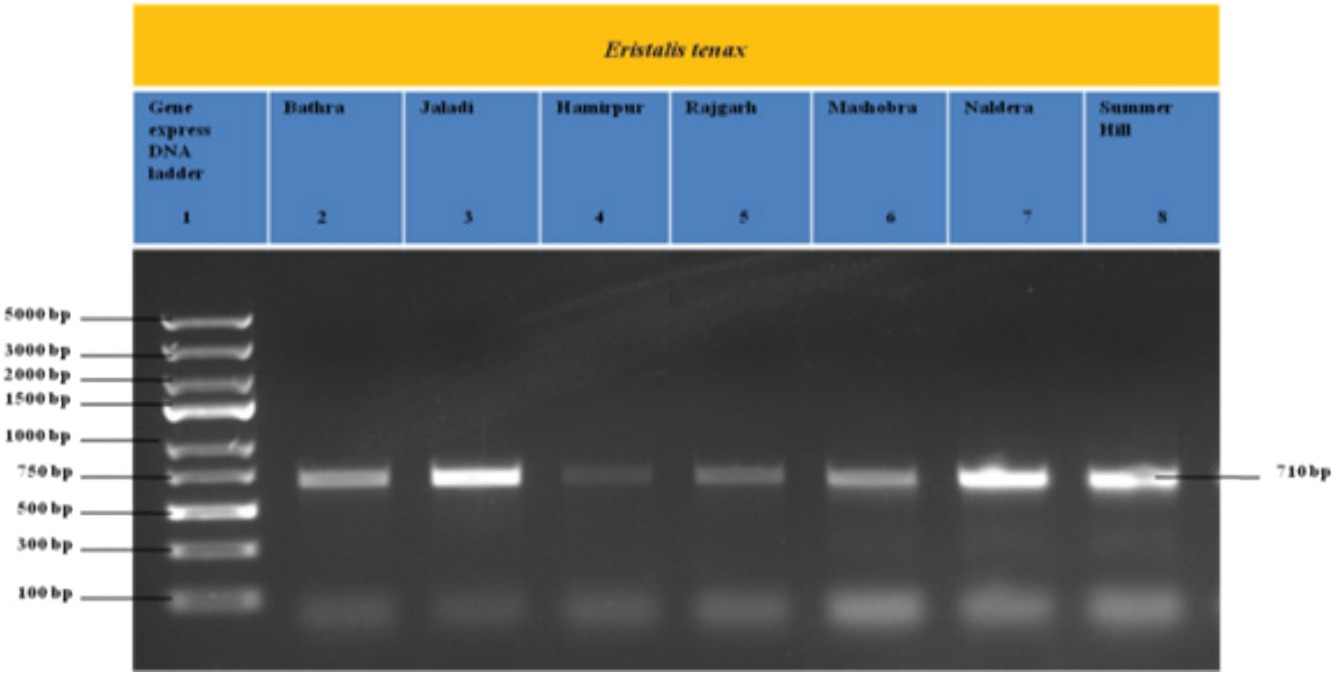

Figure 1: Analysis of amplified PCR product in 1.2% agarose: Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5, 6, 7, 8: 710 bp size mtCOI gene.

Table 2: Frequency percentage (%) of transitions and transversions and transition/transversion ratio (Ts/Tv) of COI gene.

Transition (%)					Transversion (%)								Ts/Tv Ratio
COI Gene	G/A	C/T	T/C	A/G	A/T	A/C	T/A	T/G	C/A	C/G	G/T	G/C	2.56
	39.32	12.32	5.2	18.58	4.6	1.94	3.91	1.85	3.91	1.85	4.6	1.94	

Present study showed that, molecular phylogenetics and genetic diversity among species can be useful in study the taxonomic and evolutionary relationship in different insect species. The mtCOI gene is used to study the intra-population genetic variation and also helps to study that how geographical variation changed the behavior and biology of insects [7]. Molecular markers are very helpful in determining the gene flow and genetic differences within and between insect species [8-10]. Many researchers use mtCOI gene for insect identification. Bouga et al. [11] classified the honeybee subspecies by using different molecular methods. Oldroyd et al. [12] used mtCOI gene for study the genetic divergence within species of *Apis cerana* in South India. Gaikwad et al. [13] studied the phylogenetic variation in *Apis cerana* of North Western Ghats of Maharashtra.

The present observations revealed that the percentage of A+T (69.03%) was higher than the percentage of C+G (30.97%), which showed that sequences were AT biased which were similar to the findings of Chalpathy et al. [14] who stated that honeybees sequences were AT biased and population showed natural selection from 12 localities in Karnataka. Similarly, Insuan et al. [15] studied the genetic diversity of *Apis dorsata* in Thailand and found limited genetic diversity in *Apis dorsata* samples. Present results are also accordance with the findings of Tanaka et al. [16], who studied the genetic variation of *Apis dorsata dorsata* from three different locations in Borneo and observed genetic variability among sequences of *Apis dorsata dorsata* of Borneo. In a similar study, Rukhsana et al. [17] studied the phylogenetic relationship of *Apis cerana* from Kerala using cytochrome oxidase subunit I gene (COI) and found **significant variation in *Apis* species.**

Significance of Study

Global climate change altered the intraspecific genetic diversity which is responsible for evolutionary changes and helps the species to adjust according to the new changing environmental conditions [18]. Sometimes, these variations will limit genetic diversity in populations and species, leading to population viability and extinction in extreme cases. To reduce the risk of extinction of species and ecosystems there is need of further characterization of species at species and subspecies level [19,20].

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OL589638      ------------------------------------------------------------  0
OK444106      ------------------------------------------------------------  0
OK655835      --------------GTAGGAACTTCCTTAAGAATTTTAATTCGAGCTGAATTAGGCCATC  46
OL589625      --------------------------------AATTTTAATTCGAGCTGAATTAGGCCATC  29
OK465102      CATGAGCAGGTATAGTAGGAACTTCCTTAAGAATTTTAATTCGAGCTGAATTAGGCCATC  60
OK559908      ----------TATAGTAGGAACTTCCTTAAGAATTTTAATTCGAGCTGAATTAGGCCATC  50
OQ359958      ----------TATAGTAGGAACTTCCTTAAGAATTTTAATTCGAGCTGAATTAGGCCATC  50

OL589638      ------------------------------------------------------------  0
OK444106      -----GCATTAATTGGAGATGATCAAATTTATAATGTTATTGTAACAGCTCATGCCTTTG  55
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OL589625      CTGGAGCATTAATTGGAGATGATCAAATTTATAATGTTATTGTAACAGCTCATGCCTTTG  89
OK465102      CTGGAGCATTAATTGGAGATGATCAAATTTATAATGTTATTGTAACAGCTCATGCCTTTG  120
OK559908      CTGGAGCATTAATTGGAGATGATCAAATTTATAATGTTATTGTAACAGCTCATGCCTTTG  110
OQ359958      CTGGAGCATTAATTGGAGATGATCAAATTTATAATGTTATTGTAACAGCTCATGCCTTTG  110

OL589638      -------------------------TATTATAATTGGAGGATTTGGAAATTGATTAGTTC  35
OK444106      TAATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTC  115
OK655835      TAATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTC  166
OL589625      TAATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTC  149
OK465102      TAATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTC  180
OK559908      TAATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTC  170
OQ359958      TAATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGATTTGGAAATTGATTAGTTC  170
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OK444106      CTCTTATATTAGGAGCCCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTCTGAT  175
OK655835      CTCTTATATTAGGAGCCCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTCTGAT  226
OL589625      CTCTTATATTAGGAGCCCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTCTGAT  209
OK465102      CTCTTATATTAGGAGCCCC**C**GATATAGCATTTCCTCGAATAAATAATATAAGTTTCTGAT  240
OK559908      CTCTTATATTAGGAGCCCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTCTGAT  230
OQ359958      CTCTTATATTAGGAGCCCCTGATATAGCATTTCCTCGAATAAATAATATAAGTTTCTGAT  230
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OK444106      TATTACCTCCTTCTTTAACATTATTATTAGTAAGAAGTATAGTAGAAAATGGAGCTGGAA  235
OK655835      TATTACCTCCTTCTTTAACATTATTATTAGTAAGAAGTATAGTAGAAAATGGAGCTGGAA  286
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OK559908      TATTACCTCCTTCTTTAACATTATTATTAGTAAGAAGTATAGTAGAAAATGGAGCTGGAA  290
OQ359958      TATTACCTCCTTCTTTAACATTATTATTAGTAAGAAGTATAGTAGAAAATGGAGCTGGAA  290
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OL589638      CAGGATGAACTGTATACCCACCTTTATCAAGTAACATTGCACACGGAGGAGCCTCAGTTG  215
OK444106      CAGGATGAACTGTATACCCACCTTTATCAAGTAACATTGCACACGGAGGAGCCTCAGTTG  295
OK655835      CAGGATGAACTGTATACCCACCTTTATCAAGTAACATTGCACACGGAGGAGCCTCAGTTG  346
OL589625      CAGGATGAACTGTATACCCACCTTTATCAAGTAACATTGCACACGGAGGAGCCTCAGTTG  329
OK465102      CAGGATGAACTGTATACCCACCTTTATCAAGTAACATTGCACACGGAGGAGCCTCAGTTG  360
OK559908      CAGGATGAACTGTATACCCACCTTTATCAAGTAACATTGCACACGGAGGAGCCTCAGTTG  350
OQ359958      CAGGATGAACTGTATACCCACCTTTATCAAGTAACATTGCACACGGAGGAGCCTCAGTTG  350
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OK444106      ATTTAGCAATTTTTTCACTTCATTTATCTGGTATATCATCTATTTTAGGTGCAGTAAATT  355
OK655835      ATTTAGCAATTTTTTCACTTCATTTATCTGGTATATCATCTATTTTAGGTGCAGTAAATT  406
OL589625      ATTTAGCAATTTTTTCACTTCATTTATCTGGTATATCATCTATTTTAGGTGCAGTAAATT  389
OK465102      ATTTAGCAATTTTTTCACTTCATTTATCTGGTATATCATCTATTTTAGGTGCAGTAAATT  420
OK559908      ATTTAGCAATTTTTTCACTTCATTTATCTGGTATATCATCTATTTTAGGTGCAGTAAATT  410
OQ359958      ATTTAGCAATTTTTTCACTTCATTTATCTGGTATATCATCTATTTTAGGTGCAGTAAATT  410
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OK559908        TCATTACAACTATTATTAATATACGATCAACAGGAATTACATATGATCGAATACCTTTAT   470
OQ359958        TCATTACAACTATTATTAATATACGATCAACAGGAATTACATATGATCGAATACCTTTAT   470
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OK444106        TTGTATGATCTGTAGGTATTACAGCTTTACTACTACTTCTTTCCCTACCAGTTTTAGCAG   475
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OK465102        GAGCAATTACTATATTATTAACTGATCGAAATTTAAATACATCATTTTTTGATCCAGCAG   600
OK559908        GAGCAATTACTATATTATTAACTGATCGAAATTTAAATACATCATTTTTTGATCCAGCAG   590
OQ359958        GAGCAATTACTATATTATTA----------------------------------------   550
                ********************

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OK444106        GAGGAGGTGATCCAATTTTATACCAACATTTATTTTGA-------  573
OK655835        GAGGAGGTGATCCAATTTTATACCAACATTTATTTTGATTTTTTG 631
OL589625        GAGGAGGTGATCCAATTTTATACCAACATTTA-------------  601
OK465102        GAGGAGGTGATCCAATTTTATACCAACATTT--------------  631
OK559908        GAGGAGGTGATCCAATTTTATACCAACATTTATTTT---------  626
OQ359958        ----------------------------------------------  550
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Figure 2: CLUSTAL Omega (1.2.4) multiple sequence alignment of COI sequences of *Eristalis tenax* from different altitudes of Himachal Pradesh.

Table 3: Mean frequencies (%) for base compositions at different codon positions for COI region.

Samples	First codon				Second codon				Third codon				Total (%)	
Eristalis tenax	T	C	A	G	T	C	A	G	T	C	A	G	C+G	A+T
OL589638, Mashobra	41.2	26.7	13.9	18.2	41.2	5.5	53.3	0	29.7	15.8	27.9	26.7	30.96	69.06
OK655835, Summer Hill	42.9	25.2	14.8	17.1	42.4	6.2	51.4	0	28	15.2	28	28.9	30.86	69.16
OL589625, Rajgarh	42.5	25.5	15.5	16.5	41.8	6	51.7	0.5	26.5	16	28.5	29	31.16	68.83
OQ359958, Naldera	44.8	25.7	12.6	16.9	41.3	6.5	52.2	0	26.2	14.8	30.1	29	30.96	69.06
OK444106, Bathra	42.9	25.7	15.2	16.2	42.4	5.8	51.8	0	27.7	15.2	28.8	28.3	30.39	69.59
OK559908, Hamirpur	42.8	25.5	14.9	16.8	42.1	6.2	51.7	0	27.3	15.3	28.7	28.7	30.82	69.16
OK465102, Jaladi	41.7	26.1	14.7	17.5	41	6.7	52.4	0	26.7	15.2	28.6	29.5	31.66	68.36
Average	**42.7**	**25.7**	**14.5**	**17**	**41.8**	**6.1**	**52**	**0.07**	**27.4**	**15.3**	**28.6**	**28.6**	**30.97**	**69.03**

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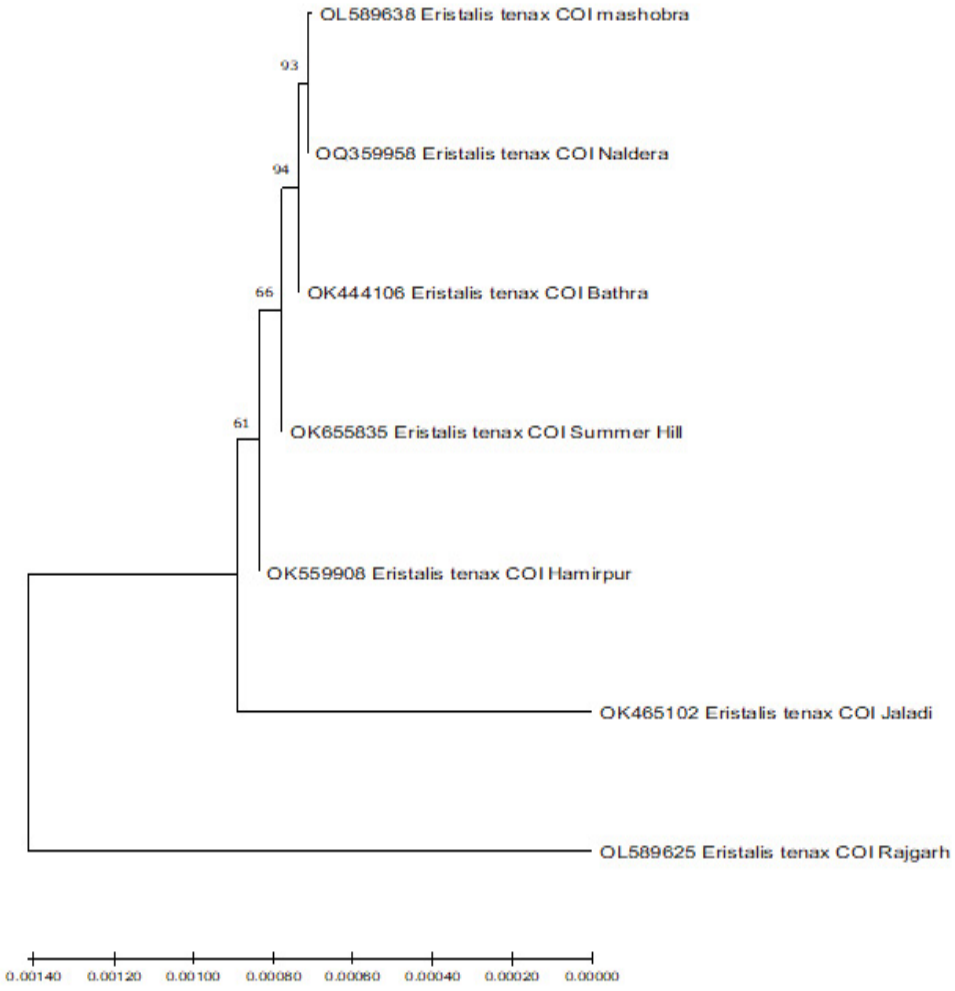

Figure 3: Phylogenetic tree of *Eristalis tenax* showing genetic relationships derived from COI sequence by using Neighbor-Joining method of MEGA X Software.

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Conflicts of Interest

The author declares no conflict of interest in the publication of this work.

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