Chapter IV

Phylogenetic Correlation and Interaction of Host Plant and Pest Species with Special Reference to Coleoptera

4. 1 Introduction

Plant chemistry has played a significant role in the evolution of host shifts by phytophagous insects. Identifying the causes that have facilitated macroevolutionary-scale host changes by phytophagous insects is crucial to understanding plants and insects' interactions. For more than the last 30 years, identifying the influences driving the nature of plants' relationships and phytophagous insects has been a critical interest in plant-insect interactions. At the macroevolutionary level, the herbivorous insects' shifts are mediated by the secondary planate chemical similarity or allopatric cospeciation (Wink, 2018). The barcoding of interactions between host plants and insects has made considerable progress in the last few years (Matheson *et al.,* 2008). The majority of the pioneering studies in this field were performed on Coleoptera (Jurado-Rivera *et al.,* 2009; Navarro *et al.,* 2010; Kitson *et al.,* 2013; García-Robledo *et al.,* 2013; Kishimoto-Yamada *et al.,* 2013) and Orthoptera (Ibanez *et al.,* 2013; Avanesyan, 2014).

Diverse methods were used to collect data on host relationships and insects' host preferences, but they are both time-consuming and have different limitations. Classical methods include observations of host use either in-situ (Barone, 2002) or in laboratory tests (Novotny and Weiblen, 2005 ; Novotny *et al.,* 2007; Giron *et al.,* 2018), transplantation experiments or behavioural tests by exposure to plant volatiles (Fernandez *et al.,* 2006; Chen and Fadamiro, 2007). Other studies have attempted the direct identification of the feeding source, either through morphological analysis of the gut content (Novotny *et al.,* 2007), diet plant tissue-specific staining techniques (Lewinsohn and Roslin, 2008), or diet plant isotope analysis from gut contents (Post, 2002; Navarro *et al.,* 2010)

Ehrlich and Raven, (1964) suggested that herbivorous insect and their host plants co-evolve, leading to the genesis of novel plant defences followed by the origin of specialized herbivores able to overcome the enhanced protection. Although some well-studied examples are showing tight co-evolution and co-cladogenesis (Cruaud *et*

al., 2012) the majority of plant-insect interactions result from diffuse co-evolution between plants and insect assemblages (Janz, 2011), where host switches are standard even in the systems with high consumer specialization (Wilson *et al.,* 2012). As a result, host plant defensive traits tend to be better predictors of insect community composition than host phylogeny per se, although plant traits governing insect food choice often differ among herbivores (Barbour *et al.,* 2015).

There is a dramatically growing recognition of the strong imprint left by evolutionary history on the features and evolutionary trajectories of organisms and ecological communities (Mitter *et al.,* 1991). This shift in perspective has coincided with advances in phylogenetic methodology and the existence of an abundance of new data from molecular systematics. Phylogenetic analyses can provide essential evidence on rates and patterns of character evolution and diversification, yielding new insights into evolutionary and ecological processes.

The outcome of interactions between plants and their enemies depends on both organisms (Carmona *et al.,* 2011; Antonovics *et al.,* 2013) Plant characteristics also present a phylogenetic pattern where close relatives are more likely to have identical characteristics (Swenson and Enquist, 2007). Many traits important in plant-enemy interactions show such phylogenetic signals (Agrawal, 2007; Agrawal *et al.,* 2009; Cruaud *et al.,* 2012) although exceptions (Loiola *et al.,* 2012). Suppose a plant pathogen or parasite has the requisite characteristics to circumvent or prevent such defenses and successfully target a specific plant species. In that case, it may be best able to target closely related plant species with the same defensive features and are phylogenetically preserved. Indeed, the host ranges of most plant pests and pathogens show a clear phylogenetic signal, where the probability that two plant species will share a particular pest declines steadily with the phylogenetic distance between them.

Information currently available regarding plants' interaction with pests or pathogens, or even insects, is scarce and mainly pertains to a few species. However, information on the number of known hosts and the phylogenetic divergence between known hosts and other vital species can be used to draw inferences about the plant-taxon relationships at different taxonomic or phylogenetic levels (Gilbert *et al.,* 2012) Predicting biological events and invasions is a challenge; meeting this challenge will

yield the ability to predict the organisms' ecological and developmental processes and natural or engineered ecosystem alteration. Combining information from the pest species' ecological and phylogenetic relationships and distributions of known and potential hosts offer an opportunity to map the risk of problems even before a new pest is recognized. This process could also be used to anticipate species invasions or outbreaks of disease related to novel pathogens (Worner and Gevrey, 2006).

The creation of forecasts of conflict or contamination requires two essential steps: the first is to estimate the probability of exchanging a pest from basic linear regression (Gilbert *et al.*, 2012). The second is to use the possibility to determine the trends of contact distribution, considering the species' existing hosts, or those that may theoretically interfere with the species, including their phylogenetic similarity to the interacting agents (i.e., vectors, rodents, etc.). The first step of this process' sows estimatiility of sharing a host, considering different phylogenetic proximity levels among hosts and host range size. Therefore, this result is very informative from an ecological and evolutionary perspective in evaluating patterns of infection (Parker *et al.*, 2015). The second step projects the likelihood of interaction over space, presenting spatially explicit predictions of the agents and their hosts' interaction.

In the present analysis, an attempt is made to measure the likelihood of a source host sharing a pest with a target host, considering the phylogenetic difference between them, and using this information to estimate the strength of the predicted risk relation of the plant to Coleoptera pests. This procedure represents an efficient method for performing a plant assessment, especially when interpretation depends on host plants' pest interactions. We apply this protocol to a set of coleopteran pests that depicted maximum infestation and their associated hosts to illustrate this assessment's efficacy and evaluate its similarity to empirical evidence.

4. 2 Materials and Methods

Procedure for DNA isolation from the plant

The DNA isolation from the plant has been extracted by the method (Edwards *et al.*, 1991).

The steps that may be included in plant DNA preparation are as followed. Components of the process may include isolation of specific tissue, grinding (or other mechanical disruption), extraction into solution, solvent purification, and precipitation.

Components of DNA extraction solutions

- **Buffer:** Buffers are used to control the pH of the extraction solution.
- **Salts:** Salts may influence the solubility of DNA and other molecules in the extraction.
- **Chelating agents:** In the extraction solution, chelating agents such as EDTA bind metal ions.
- **Detergents:** Detergents assist in tissue destruction. The plant DNA extraction protocols employed several different detergents.
- **Phenolic binding agents**: Defense against phenolics also involves PVP (Kim and Masuda, 1997). Added citric acid to the extracts to avoid polyphenolics production.
- **Enzymes**: Ribonuclease for eliminating contaminating RNA is the most common enzyme use in DNA isolation procedures.
- **Solvents:** MiliQ Water is the solvent used in almost all protocols.

Tissue disruption

- **Mechanical:** Physical tissue destruction allows for the introduction of DNA into the solution for extraction. Excess mechanical perturbation causes DNA shearing. Ideally, only slight mechanical action is used to extract the DNA from the tissue. Tough plant tissues may be challenging targets for high molecular weight DNA isolation.
- **Heating:** Heating contributes to tissue destruction, particularly at high temperatures (e.g., boiling).

High throughput extraction methods

1. The tissue was macerated using sterilized mortar and pestle at room temperature, without buffer, for 15 seconds.

2. Then 400 µl of extraction buffer (200 mM Tris HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added and the samples were vortexed for 5 seconds.
3. This mixture was then left at room temperature until all the samples were digesting properly (> 1 hour).
4. The extracts were then centrifuged at 13,000 rpm for 1 minute, and 300 µl of the supernatant transferred to a fresh Eppendorf tube.
5. This supernatant was mixed with 300 µl isopropanol and left at room temperature for 2 minutes. Followed by centrifugation at 13,000 rpm for 5 minutes
6. The pellet was supernatant, discarded, and the pellet was allowed to dry, followed by dissolved in 100 µl 1 x TE.
7. This DNA was stored at -20 ◦c for the further process.

The integrity of DNA was checked by using 0.8% agarose gel electrophoresis. The isolated DNA samples were quantified to find out the amount of DNA using a Nanodrop Spectrophotometer. The quantification was done taking the A_{260}/A_{280} ratio, as it reveals contaminants' presence and gives evidence of possible degradation. An A_{260}/A_{280} ratio of 1.8 was considered acceptable for DNA.

The DNA product was then amplified for trnL using PCR (prima-96, HiMedia, India) and primer, as shown in Table 4.1.

PCR primers for trnL:

Name of DNA marker and primer	**Primer sequence (5' to 3')**	**Reference**
trnL intron		
c A49325	5'CGAAATCGGTAGACGCTACG'3	Taberlet*et al.*, 1991
d B49863	5'GGGGATAGAGGGACTTGAAC'3	Taberlet*et al.*, 1991

Table 4. 1: PCR primers for trnL

PCR Conditions:

The DNA product was then amplified in PCR for trnL at 60°C denaturation for 3 min, 27 cycles of 94°C for 1:00 min, 42 °C annealing for 00:30 min, 72°Cfor 1:00 min, followed by 35 cycles of 94°C for 1:00 min, 50 °C for 1:30 min and 72 °C for 1:00 min, and extension was carried out 72 °C for 7 min. A total of 27 cycles were performed using primers (Table 4. 2).

Stage 1 (1 cycle)	Stage 2 (27 cycles)	Stage 3 (1 Cycle)
60°C - 3 min.	94°C -60 sec	72 °C-5 min
	42°C - 30 sec	4°C stop for ∞ time
	72 °C- 60 sec	

Table 4. 2: PCR conditions for trnL of the plant species

Electrophoresis of PCR reactions

Amplification of DNA was then checked by running the samples on 2% agarose gel using 680bp DNA ladder and visualized in gel dock. The amplified products were then sent to commercial sequencing at Chromos Biotech Pvt. Ltd. and Eurofins Pvt Ltd, Bangalore, India, where the chain termination method was used for sequencing.

Sequencing

Sequencing was carried out using the Sanger sequencing method, as described in chapter III.

Homology Analysis

The sequence obtained carried out blast using NCBI/BOLD system, and the minimum distance was calculated using neighborhood analysis.

Phylogenetic Analysis

Also, the similarity of common pest and its associated host plant was subjected to tree construction using the Maximum Likelihood method where the test of phylogeny was performed using the bootstrap method with 500 replicates to nucleotide type substitution. The whole composite probability approach was used to determine homology, and complete deletion was used to achieve the complete sequence. This was accomplished through MEGA X software.

Bioinformatics Analysis

- %GC content analysis was done for each sequence.
- AT and GC skew analysis

The sequenced data were subjected to strand asymmetric analysis, which was calculated using the formula(s) AT-skew = (A−T)/(A C T), GC skew = (G−C)/(G C C).

Rate of Synonymous and Non-Synonymous Probability

Using the Nei-Gojobori system, the numbers of synonymous and non-synonymous variations between sequences is determined. The significance for noted at $p<0.05$ for the synonymous and non-synonymous occurrence of nucleotides.

4. 3 Result

A total of 51 insect pest species belonging to 4 orders were sequenced from the pest species (Chapter III), from which 13 polyphagous Coleoptera pest species were chosen for the genetic correlation with crops and vegetables.

Insect – Host Plant	AT%	GC%	AT-Skew	GT - Skew	Evolutionary analyses	Test of Neutrality
Altica cyanea - MT707358 *Lagenaria siceraria* (Brinjal)	64.81	35.19	-0.07	-0.11	6.96	1.00
Aulacophora indica - MT863614 *L. siceraria* (Brinjal)	64.62	35.38	0.01	-0.06	4.57	0.26
Chiloloba orientalis - MT707357 *Oryza sativa* (Rice)	67.16	32.84	-0.28	0.08	8.79	1.00
Lanelater fuscipes - MT547190 *Daucus carota* (Carrot)	57.49	42.51	0.02	-0.11	2.79	0.46
Monolepta signata - MT707359 *Triticum monococcum* (Wheat)	69.16	30.84	0.02	0.08	7.06	0.21
Mylabris pustulata - MT863613 *Solanum melongena* (Brinjal)	65.58	34.42	0.03	-0.03	7.87	0.456
Myllocerus dorsatus - MT863617 *Abelmoschus esculentus* (Okra)	62.9	37.10	-0.01	0.10	9.48	0.0006
Oxycetonia versicolor - MT707356 *Luffa aegyptiaca* (Sponge gourd)	68.52	31.48	-0.12	0.02	5.57	0.0888
Protaetia aurichalcea - MT863616 *L. siceraria* (Brinjal)	64.21	35.79	-0.081	-0.13	5.82	0.145
Sitophilus oryzae - MT731601 *Zea mays* (Maize)	65.18	34.82	0.047	-0.02	4.49	0.0677

Table 4. 3: Homological insect – host plant with its AT- GC content, AT-GC- Skew, evolutionary analyses and test of neutrality

Altica cyanea shared the homology with *L. siceraria*, *Spinacia oleracea* and *Oryza sativa* indica however, maximum homology was exhibited with *L. siceraria* (Figure 4. 1).

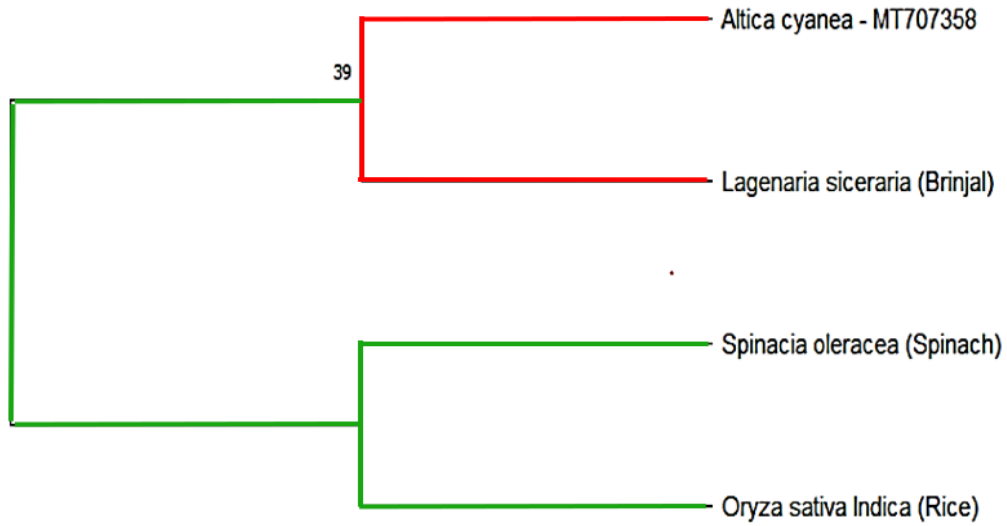

Figure 4. 1: Homology of *Altica cyanea* with its host plants

A genomics analysis AT content of *A. cyanea* (66.58%) and *L. siceraria* (63.72%) and GC content of *A. cyanea* (333.41%) and *L. siceraria* (36.28%) was found to be the average of 64.81% and 35.19%, respectively.

Further, the closely related pest – host sequences were analyzed for AT and GC skews, where AT skew was revealed that *A. cyanea* (-0.07) and *L. siceraria* (-0.07) with the average of -0.11 whereas GC skew has resulted that *A. cyanea* (-0.07) and *L. siceraria* (-0.11) with the average of -0.07.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *A. cyanea, L. siceraria* and *Spinacia oleracea*. The nucleotide frequencies for COI were 29.59% (A), 35.09% (T/U), 21.04% (C), and 14.27% (G). The transition/transversion rate ratios were k_1 = 6.928 (purines) and k_2 = 5.295 (pyrimidines). The overall transition/transversion bias is R = 2.776, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Codon-based Test of Neutrality analysis between sequences was performed for *A. cyanea and L. siceraria* (1), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences.

There were 199 positions in the final dataset, and there was no significant ($p<0.05$) synonymous and non- synonymous rate determined between the species (Table.4.3)

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 61.40, 61.29 and 71.78%, respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 38.60, 38.71 and 28.25%, respectively, the percentage was calculated. The pairwise distance among insect-plant species was calculated to be 6.96 for COI (Table 4.3).

- ***Altica cyanea* - MT707358**

ATAATTTTCTTTATAGTAATACCCATAATAATTGGAGGTTTTGGAAACTGATTAGTCCCTTT
AATAATTGGGGCCCCTGATATAGCTTTCCCTCGAATAAATAATATAAGATTCTGGTTACTC
CCGCCATCAATTTTTTTATTATTAATAAGAAGGTTAACAGAAAGAGGAGCAGGAACTGGA
TGAACGGTTTATCCCCCCCTATCATCCAATCTTGCCCATAATGGGCCATCTGTGGATTTAG
CTATTTTTAGCCTTCATTTAGCAGGAATTTCATCAATTCTAGGTGCTATTAATTTTATTACT
ACAATAATCAATATACGACCTAAAGGAATATCTATAGATCAAATACCTTTATTTGTATGAG
CTGTCCTTATTACAGCAATTCT

- **AB935651.1 *Spinacia oleracea* (Spinach)**

AATTGGCTTGAGCCTTAGTATGGAGACCTACTAAGTGAAAACTTTCAAATTCAGAGAAAC
CCTGGAATTAATAAAAATGGGCAATCCTGAGCCAACTCCTTTTTTCAAAAGCAAGAATAA
AAAAAAAGGATAGGTGCAGAGACTCAAAGGAAGCTGTTCTAACAAATATTCATTCACCAA
ATAATTCACTCCATAGTCTGATAGATCTTTGGGAGGAACTTAATTAATATTAATCGGACGA
GAATAAAGATAGAGTCCTGTTCTACATGTCAATACTGACAACAATGAAATTTATAGTAAG
AGGAAAATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAGAGT
TTTTTACTTCCTAATTACTTCTCTTTTTTTTCATTAACGGGTTGAAGGCGGTTATATCCCTAA
TTTTCTCTTTTCACAAGTCTTGTGATATATATGATAAACATACAAATAAATATTTTTGAATA
AGGAGTACTCAGTTTAGTGATTCACAATTCATATTATTACTTGTACTAAAACTTAAATAAA
ATTATAGACAAATTTGGAAAACTCAAGGGATTCCGGTACGCATATAATACTTTGTAATACT
TTTCATCCGTTTAATTGACATAAACCCGAGTTATCGAGTAAAATGAAATGAGAAGATGAT
GCGCCCGAAAGGTGAA

- **AY792515.1 *Oryza sativa* (Rice)**

GGTATGGAAACCTGCTAAGTGGCAACTTCCAAATTCAGAGAAACCCTGGAATTAAAAAAG
GGGCAATCCTGAGCCAAATCCATGTTTTGAGAAAACAAGCGGTTCTCGAACTAGAACCCA
AAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCGAGTTAATTAC
GTTGTGTTGCTAGCGGAACTCCCTTCTAAATTAGGGAAAGAAGGGCTTTCGAAATCTAATA
CACACGTATAGATACTGGCATAGCAACGATTAATCACAGAACTCATATCATAATATAGGT
TCTTTAATTCTTTTTTAAAATGAAAATAGGAATGATTATGAAATAGAAAATTCATAATTTT
TTTAGAATTGTTGTGAATCCATTCCAATCGAATATTGAGTAATCAAATCCTTCAATTCATA
GTTTTCGAAATCTTTTTAAAAGCGGATTAATCGGACGAGGATAAAGAGAGAGTCCCATTC
TACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGTCGACTTTCTA
AGTCGTGAG

- ***Lagenaria siceraria* (Brinjal)**

TAGCAATTCACATTATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAAT
TTGATTTCTTATCTGTCTACTGTACTGAAACTTCCAAAGTCTTATCCACACAAGGCTTGTGG
TATATATTCTATATGATACACGTACAAACGAACATCCTTGCGCAAGTAATCCCCCTTGTGA
AATTTGAATGATTAACAATAAGCCCTGAAATTTCGTGGATCTTCAAAAAGAAGACTTTGG

AATATCCTCTTATTTACAATTGAGGCCCGTTGGCCTCTTTAATCATTTATCCTTGCATCATA
GACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGGCCGGGATAGCTCAGTT
GGTAGAGCAGAGGACCGTTGGCCTCTTTAATTATTCAAACGAACATCCTTGCGCAAGTAA
TCCCCCTTGTGAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTC
TTATCCAAGCCCTGAAATTTCGTGGATCTTCAAAAATATCCTTTCATTAGCAATTCACATT
ATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAATTTGATTTCTTATCA
CAAGGCTTGTGGTATATATTCTA

Aulacophora indica shared the homology with *L. aegyptiaca*, *Moringa oleifera*, and *L.siceraria* however, maximum homology was exhibited with *L. siceraria* (Figure 4.2).

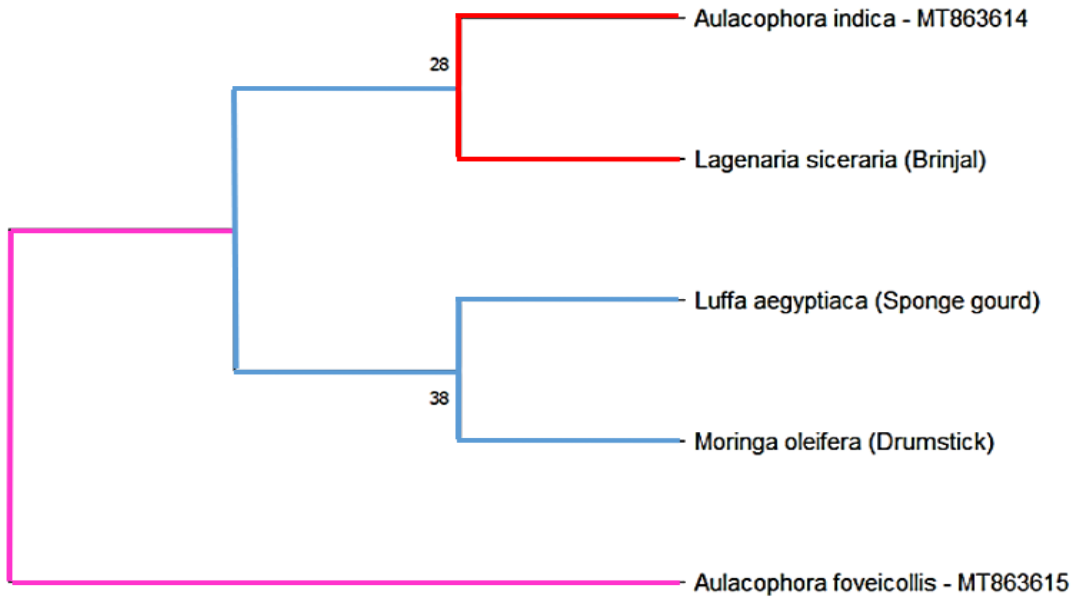

Figure 4. 2: Homology of *Aulacophora species* with its host plants

A genomics analysis AT content of *A. indica* (66.58%) and *L. siceraria* (63.72%) and GC content of *A. indica* (33.4%) and *L. siceraria* (36.28%) resulted with the average of 64.81% and 35.19%, respectively.

Further, the closely related pest – host sequences were analyzed for AT and GC skews, where AT skew was revealed that *A. indica* (-0.065 and *L. siceraria* (-0.069) with the average of -0.067 whereas, GC skew has resulted that *A. indica* (-0.09) and *L. siceraria* (-0.11) with the average of -0.105.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *A. indica, L. siceraria* and *L. aegyptiaca*. The nucleotide frequencies for COI were 35.46% (A), 28.77% (T/U), 17.42% (C), and 18.35% (G). The transition/transversion rate ratios were k_1 = 4.543 (purines) and k_2 =

4.963 (pyrimidines). The overall transition/transversion bias was R = 2.19, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Codon-based Test of Neutrality analysis between sequences was performed for *A. indica and L. siceraria* (0.26), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There were 202 positions in the final dataset, and there was no significant ($p<0.05$) synonymous and non- synonymous rate determined between the species (Table 4.3) Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 60.12, 60.62and 73.13% respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 39.88, 39.38and 26.88% respectively percentage was calculated. The pairwise distance among insect-plant species was found to be 4.7 for COI (Table 4. 3).

- ***Aulacophora indica*** **- MT863614**

TCTGTTAATAATATTGTAATGGCTCCAGCTAAAACTGGTAGAGATAATAATAATAATACA
GCTGTAATAACAACAGCTCATACAAATAGTGGTATTCGGTCTAGGGTTATTCCTTTAGGAC
GCATATTAATTACGGTTGTGATAAAATTAATTGCTCCTAAAATTGAAGAAATTCCGGCTAA
ATGTAAACTGAAAATTGCTAAATCAACAGAAGAACCTCCATGGGCAATATTTGAAGAAAG
AGGAGGGTACACAGTTCAACCAGTTCCAGCCCCTCTTTCAACAACTCTACTTATAATTAAT
AAAAATAGAGAAGGAGGAAGTAAT

- ***Aulacophora foveicollis*** **- MT863615**

GGAAATGATCAAATTTATAATGTAATTGTCACTGCCCATGCATTCATTATAATTTTTTTTAT
AGTTATACCAATTATAATCGGAGGGTTTGGAAACTGATTAGTACCCTTAATAATTGGGGCT
CCTGATATAGCTTTCCCTCGTATAAATAATATAAGATTTTGATTACTTCCTCCTTCTCTATT
TTTATTAATTATAAGTAGAGTTGTTGAAAGAGGGGCTGGAACTGGCTGAACTGTGTACCCT
CCTCTTTCTTCAAATATTGCCCATGGAGGTTCTTCTGTTGATTTAGCAATTTTCAGTTTACA
TTTA

- **KF487460.1** ***Luffa aegyptiaca*** **(Sponge gourd)**

GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAAT
CCTTTTTCCGAAAACAAAAAAAAGGGGTAGGTGCAGAGACTCAATGGAAGCTGTTCTAAC
AAATGGAGTTGACTACGTTGCGTTAGTAAAGGAATGAATCCTTCTATCGAAACTCCAGAA
AAGATGAAAGATAAACGTATTACGTACGGAAATACTATTTGATTAATGACAACCCGAATC
TCTATTTTTTTTTATATATATATAAATATAAAAAATGATATATAAAAATGACCGAATTGTT
ATGAATCGATTCCAATATCTCCAAGTTGAAAAAAGAATCGAATATTCATTGATCAAATCAT
TTACTCCATCATACATAGTCTGATAGATCTTTTGAAGAACTGATTGATCARATGCGAATAG
AATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAAAAATGAAATTTATAGTAAGA
GGAAAATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAACCCTA
AAAAGGCCCGTTGGCCTCTTTAATTATTTATTATCCTTTCATTAGCAATTCACAATTCGTTA
TGTTTCTCATTCATTCTACTCTTTCACAAGCGTATCTGAGCGGAAATTTGATTTCTTAGCAC
AAGACTTGTGGTATATATTTATATGATACACGTACAAACGAACATCCTTGCGCAAGGAAT
CCCCGTTGTTAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTCT
TATCCAAGCCCTGAAATTTCGTGGATCTTTAAAAAGAAGACTTTGGAATACCTTTTTTCTT

ATTTACAATTGACATAGACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGG
CCGGGATAGCTCAGTTGGTAGAGCANAGGACTGAAAATC

- ***Moringa oleifera*** **(Drumstick)**

CAAATTCAGAGAAACCCGGGAATGAAAAATGGGCAATCCTGAGCCAAATCCTGTTTTACG
AGAACAAACAAGGGTTCAGAAAGCGAGGGGATAGGTGCAGAGACTCAATGNNGAAGCCG
TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAACTTGGTATGGAAACCTACTA
AGTGATAACTTTTCCTTCTATCGAAACTCCAGAAAGGATAAACTTATATATACATACGTAT
ACGTAATGCAAAACTATCTCAAAAATGGCGATCTGAATCTTTTTTATATGAAAAAAAAGA
ATTGTTGTGAATCAATTCGAAGTTGAAGAAAAAATCGAATATTCATTGATCAAATCATTCA
CTCCATAGTCTGATAGATCTTTGCAAGAACTGATTAATCGGACGAGAATAAAGATAGAGT
CCCATTCTACATGTCAATACCGACAACAATGCAATTTATAGTAAGATGAAAAAAAAAAA

- ***Lagenaria siceraria*** **(Brinjal)**

TAGCAATTCACATTATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAAT
TTGATTTCTTATCTGTCTACTGTACTGAAACTTCCAAAGTCTTATCCACACAAGGCTTGTGG
TATATATTCTATATGATACACGTACAAACGAACATCCTTGCGCAAGTAATCCCCCTTGTGA
AATTTGAATGATTAACAATAAGCCCTGAAATTTCGTGGATCTTCAAAAAGAAGACTTTGG
AATATCCTCTTATTTACAATTGAGGCCCGTTGGCCTCTTTAATCATTTATCCTTGCATCATA
GACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGGCCGGGATAGCTCAGTT
GGTAGAGCAGAGGACCGTTGGCCTCTTTAATTATTCAAACGAACATCCTTGCGCAAGTAA
TCCCCCTTGTGAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTC
TTATCCAAGCCCTGAAATTTCGTGGATCTTCAAAAATATCCTTTCATTAGCAATTCACATT
ATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAATTTGATTTCTTATCA
CAAGGCTTGTGGTATATATTCTA

Chiloloba orientalis shared the homology with *Zea mays, Cenchrus americanus, Triticum monococcum* and *Oryza sativa* however maximum homology was exhibited with *O. sativa* (Figure 4. 3) .

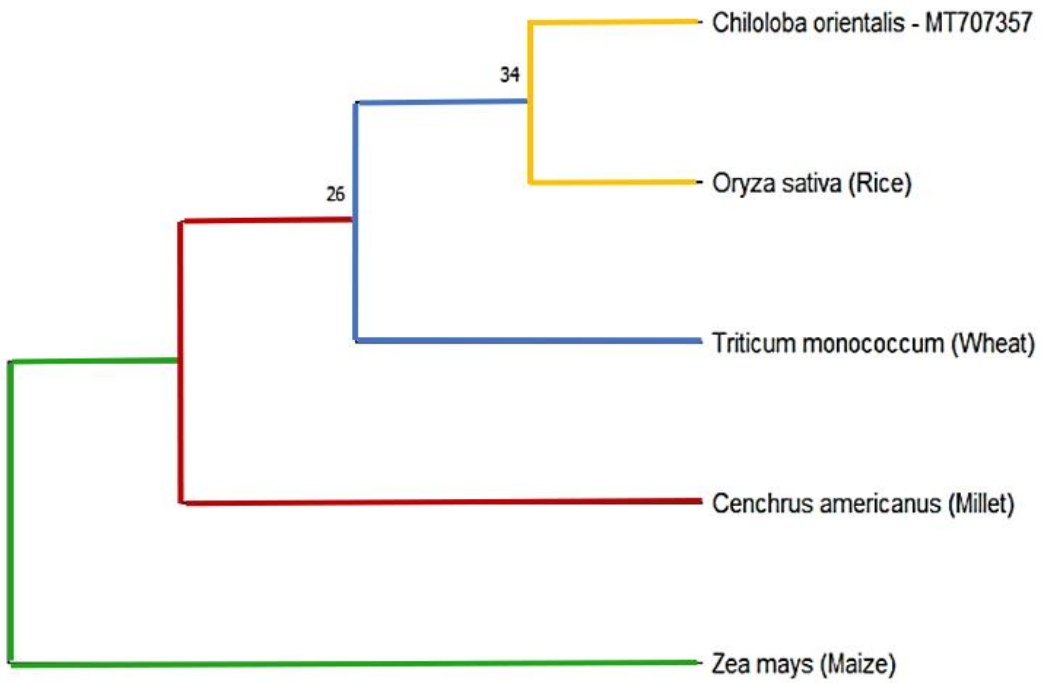

Figure 4. 3: Homology of *C. orientalis* with its host plants

A genomics analysis AT content of *C. orientalis* (65.27%) and *O. sativa* (67.74%) and GC content of *C. orientalis* (34.75%) and *O. sativa* (32.25%) was computed with the average of 67.16% and 32.84%, respectively.

Further, the closely related pest – host sequences were analyzed for AT, and GC skews, where AT skew was revealed that *C. orientalis* (-0.136) and *Oryza sativa (*-0.33) with the average of -0.284, whereas GC skew resulted that *C. orientalis* (-0.023) and *O. sativa* (0.11) with the average of -0.077.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *C. orientalis, O. sativa* and *T. monococcum*. The nucleotide frequencies for COI were 29.27% (A), 36.94% (T/U), 15.61% (C), and 18.18% (G). The transition/transversion rate ratios were k_1 = 5.715 (purines) and k_2 = 6.212 (pyrimidines). The overall transition/transversion bias was R = 2.656, where R = $[A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$.

Codon-based Test of Neutrality analysis between sequences was performed for *C. orientalis and O. sativa* (1), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences.

There was a total of 375 positions in the final dataset, and there was nonsignificant ($p<0.05$) synonymous and non- synonymous rate was determined between the species (Table 4. 3)

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 61.296, 77.92and, 62.27% respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 38.70, 22.08and 37.73% respectively percentage was calculated. The pairwise distance among insect-plant species was calculated to be 8.79 for COI (Table 4. 3).

- ***Chiloloba orientalis* - MT707357**

ATAATTTTCTTCATAGTGATGCCAATTATAATTGGCGGATTTGGTAATTGGCTTGTTCCTCT
AATACTAGGGGCCCCAGATATAGCCTTTCCTCGAATAAATAACATAAGATTTTGATTATTA
CCCCCGTCATTAACTTTACTTTTAATGAGTAGAATAGTTGAAAGAGGTGCTGGAACAGGAT
GAACAGTATACCCCCCTCTTTCTAGAAATATCGCCCATAGAGGTGCCTCTGTTGATTTAGC
TATTTTCAGTTTACATTTAGCTGGAATCTCTTCTATTTTAGGAGCTGTTAATTTTATTACTA
CTGTTATTAACATACGATCAACAGGAATAACATTTGATCGAATACCTTTATTTGTTTGATC
TGTAGCTTTAACTGC

- **DQ131551.1 *Zea mays***

CGAAATCGGTAGACGCTACGGACTTGATTGTATTGAGCCTTGGTATGGAAACCTGCTAAG
TGGTAACTTCCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATC
CCTTTTTTGAAAAACAAGTGGTTCTCAAACTAGAACCCAAAGGAAAAGGATAGGTGCAGA
GACTCAATGGAAGCTGTTCTAACGAATCGAAGTAATAACGATTAATCACAGAACCCATAT
TATAATATAGGTTCTTTATTTTATTTTTAGAATGAAATTAGGAATGATTATGAAATAGAAA
ATTCATAATTTTTTTTTAGAATTATTGTGAATCTATTCCAATCAAATATTGAGTAATCAAAT
CCTTCAATTCATTGTTTTCGAGATCTTTTAATTTTTAAAAGTGGATTAATCGGACGAGGAT
AAAGAGAGAGTCCCATTCTACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGA
AAATCCGTCGACTTTATAAGTCGTGAGGGTTCAAGTCCCTCTATCCCC

- **EU939991.1 *Pennisetum glaucum* (Millet)**

GATTGTATTGAGCCTTGGTATGGAAACCTGCTAAGTGATAACTTCCAAATTCAGAGAAAC
CCTGGAATGAAAAATGGGCAATCCTGAGCCAAATCCCTTTTTTGAAAAAACAAGTGGTTC
TAAAACTGGAACCCAAAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAC
GAATCGAAGTAATTACGTTGTGTTGGTAGGGGAACTCCCTCGAAATACTAGAAAGAAGGG
CTTTATACATTTAATACACACGTATAGATACTGACATAGTAAACGATTAATCACAGAACCC
CTATCATAATATAGGTTCTTTATTTTATATTTTTTTCTAGAATGAAATTAGGAATGATTATG
AAATAGAAAATTCTGAATTTTTTTAGAATTATTGTGAATCCATTCCAATCGAATATTGAGT
AATCAAATCCTTCAATTCATTGTTTTGAGATCAAAAGTGGATTAATCGAACGAGGATAAA
GAGAGAGTCCCATTCTACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGAAAA
TCCGTCGACTTTATAAGTCGTGAGGGTTCAAGTCCCTCTATCCCCACCCAAACCCTCTTTTA
TTCCCTGACCATAGTAGTTATCCTTTTTTTCTTTTATCAATGGGTTTAAGATTCATTAGCTTT
CTCATTCGACTCTTTCTTTCACAAAGGAGTGCGATGAGAACTCAATGAATCTTATGCTATT
CATTAAATAGAATATTTCTTTTTTATTTGATAGGACTACCCCCCCCCCGCTCCATTCCAAAT
TTGGAATGGAATACTTTATNGATTTTTTAGTCCCGTTAATTGACATAGATGCAAATACTCT
ACTAGGATGATGCNCAANAAA

- **DQ420000.1 *Triticum monococcum* (Wheat)**

ATTGAGCCTTGGTATGGAAACCTGCTAAGTGGTAACTTCCAAATTCAGAGAAACCCTGGA
ATTAAAAAAGGGCAATCCTGAGCCAAATCCGTGTTTTGAGAAAACAAGGGGTTCTCGAAC
TAGAATACAAAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCG
AGTTAATTACGTTGTGTTGTTAGTGGAATTCCTTCTAGTTCTAAATTAGAGAAAGAGGGGT
TTTATACCTTATACATTTAATAAACACGTATAGATACTGACATAGCAAACGATTAATCACA
GAACTCATATTATATTAGAATTATAATATAGGTTCTTTATCTTTTTTAAAAATGAAATTAG
AAATGATAATGATTATGAAATAAAAAACTCATATCATAATTTTTTTTTTTTCATTATTGGG
AATCCACTCCAATCGAATATTGAATAATCAAATTCTTCAATTAAAATTCAAAGTTTTCGAG
ATCTTTAAAAAAGTGGATTAATCGGACGAGGACAAAGAGAGAGTCCCATTCTACATGTCA
ATACCTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGCCGACTTTATAA

- **AY792515.1 *Oryza sativa* (Rice)**

GGTATGGAAACCTGCTAAGTGGCAACTTCCAAATTCAGAGAAACCCTGGAATTAAAAAAG
GGGCAATCCTGAGCCAAATCCATGTTTTGAGAAAACAAGCGGTTCTCGAACTAGAACCCA
AAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCGAGTTAATTAC
GTTGTGTTGCTAGCGGAACTCCCTTCTAAATTAGGGAAAGAAGGGCTTTCGAAATCTAATA
CACACGTATAGATACTGGCATAGCAACGATTAATCACAGAACTCATATCATAATATAGGT
TCTTTAATTCTTTTTTAAAATGAAAATAGGAATGATTATGAAATAGAAAATTCATAATTTT
TTTAGAATTGTTGTGAATCCATTCCAATCGAATATTGAGTAATCAAATCCTTCAATTCATA
GTTTTCGAAATCTTTTTAAAAGCGGATTAATCGGACGAGGATAAAGAGAGAGTCCCATTC
TACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGTCGACTTTCTA
AGTCGTGAG

Lanelater fuscipes shared the homology with *Zea mays*, *Solanum tuberosum*, *Triticum monococcum*, and *Daucus carota*, and maximum likelihood resulted in the closest homology were exhibited with *D.carota* (Figure 4. 4).

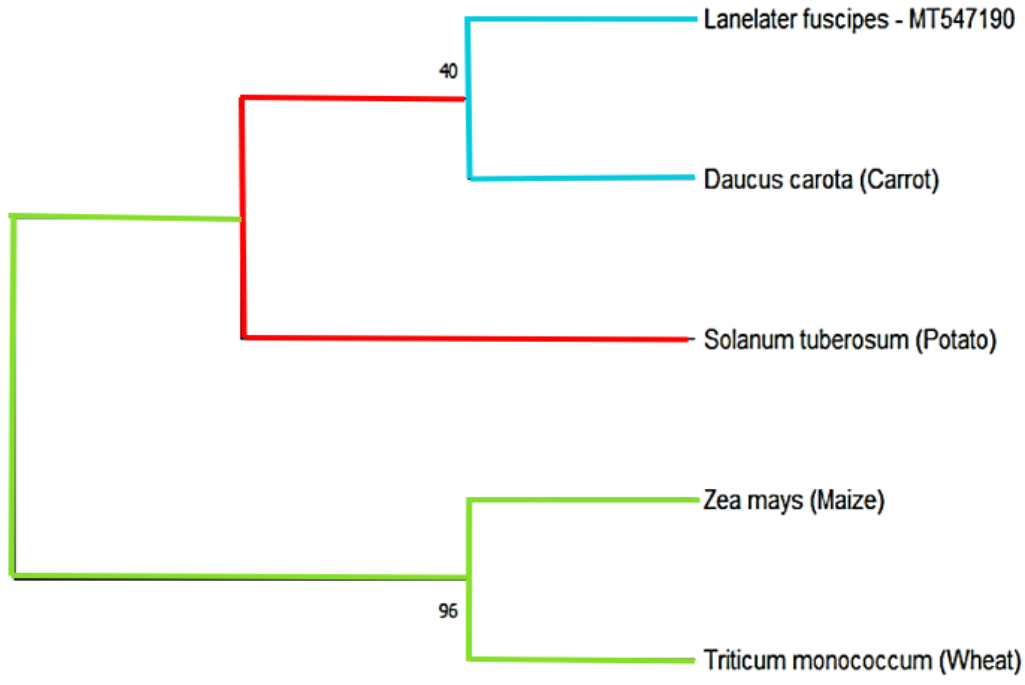

Figure 4. 4: Homology of *L. fuscipes* with its host plants

Further, the genomic analysis of AT content of *L. fuscipes* (57.25%) and *D. carota* (57.59%) and GC content of *L. fuscipes* (42.74%) and *D. carota* (42.41%) was computed with the average of 57.49% and 42.51% respectively.

Further, the closely related pest – host sequences were analyzed for AT, and GC skews, where the analysis of AT skew was revealed that at the combined level *L. fuscipes* (-0.26) and *D. carota (*-0.03) showed an average of 0.022, whereas GC skew resulted that *L. fuscipes* (-0.004) and *D. carota (*-0.03) had an average value of -0.107.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *L. fuscipes, D. carota* and *S. tubersosum.* The nucleotide frequencies for COI were 31.64% (A), 29.92% (T/U), 20.29% (C), and 18.14% (G). The transition/transversion rate ratios werek_1 = 5.957 (purines) and k_2 = 6.603 (pyrimidines). The overall transition/transversion bias was found to be R = 2.972, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$.

Codon-based Test of Neutrality analysis between sequences was performed for *L. fuscipes and D. carota* where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There were 857 positions in the final dataset, and a nonsignificant ($p<0.05$) synonymous and non-synonymous rate was determined between the species (Table 4.3)

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were found to be 53.96, 58.89and 59.61%, respectively, while that of G+C contents of the 1st, 2nd, and 3rd codons were 46.04, 41.11, and 40.39%, respectively. The pairwise distance among insect-plant species was calculated to be 2.79 for COI (Table 4.3).

- ***Lanelater fuscipes* - MT547190**

ATACTCGGAGCCCCAGACATAGCATTCCCTCGAATAAATAACATAAGATTCTGACTACTTC
CCCCATCATTATCCCTTCTTCTGATAAGAAGAATTGTTGAAAACGGAGCCGGGACCGGAT
GAACAGTCTATCCACCCCTATCAGCAAACATCGCCCATAGAGGATCTTCAGTTGACCTCGC
AATTTTTAGCCTCCATTTGGCAGGTATTTCATCCATTCTAGGAGCCGTAAATTTTATCTCTA
CTGTAATCAATATACGATCAACAGGAATTTCATTTGACCGTATACCCCTATTTGTATGAGC
TGTAGCAATTACAGCCCTTCTCCTCCTCCTATCCCTGCCGGTTCTAGCAGGAGCAATCACT
ATACTGTTAACAGATCGAAACCTAAAT

- **DQ131551.1 *Zea mays* (Maize)**

CGAAATCGGTAGACGCTACGGACTTGATTGTATTGAGCCTTGGTATGGAAACCTGCTAAG
TGGTAACTTCCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATC

CCTTTTTTGAAAAACAAGTGGTTCTCAAACTAGAACCCAAAGGAAAAGGATAGGTGCAGA
GACTCAATGGAAGCTGTTCTAACGAATCGAAGTAATAACGATTAATCACAGAACCCATAT
TATAATATAGGTTCTTTATTTTATTTTTAGAATGAAATTAGGAATGATTATGAAATAGAAA
ATTCATAATTTTTTTTTTAGAATTATTGTGAATCTATTCCAATCAAATATTGAGTAATCAAAT
CCTTCAATTCATTGTTTTCGAGATCTTTTAATTTTTAAAAGTGGATTAATCGGACGAGGAT
AAAGAGAGAGTCCCATTCTACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGA
AAATCCGTCGACTTTATAAGTCGTGAGGGTTCAAGTCCCTCTATCCCC

- **EF010973 *Solanum tuberosum* (Potato)**

GACTTAATTGGATTGAGCCTTGGTATGGAAACTTACTAAGTGATCACTTTCAAATTCAGAG
AAACCCTGGAATTAACAAAAATGGGCAATCCTGAGCCAAATCCTGTTTTCTGAAAACAAA
CAAAGGTTCAGAAAAAAAGGATAGGTGCAGAGACTCAATGGAAGCTATTCTAACAAATG
GAGTTAAATGCGTTGGTAGAGGACTCTTTACATCGAAACTTCAGAAAGAAAAAGAATGAA
GTGAAGGATAAACGTATATACATACGTATTGAATACTATATCAAATGATTAATGACGACC
CGAATCCGTAGTTTTTCTATAAAAAATCGAAGAATTGGTGTGAATCCATTCTACATTGAAG
AAAGAATCGAATATTCATTGATCAAATCATTCACTCCATAGTCTGATAGATCTTTTGAAGA
ACTGATTAATCGGACGAGAATAAAGATAGAGTCCCGTTCTACATGTCAATACCGGCAACA
ATGAAATTTATAGTAAAAGGAAAATCCGTCGACTTTAAAATCGTGAGG

- **KY697399.1 *Daucus carota* (Carrot)**

GGACTTGAACCCTCACGATTTTTAAAGTCAACGGATTTTCCTCTTACTATAAATTTCATTGT
TGCCGGTATTGACATGTAGAATGGGACTCTATCTTTATTCTCGTCCGATTAATTAGTTCTGC
AAAAGAACTATCAGACTGTGTGGTGAATGCTTTGATCAATGAATATTCGATTCTTTCTTCA
ATATGGAATCGATTCACAACAATTTTTCCCTTTTTCATGTAAAAATACAGATTCAGGTCGT
CATTAATCATTTGATAGAGTATTTCAGTACGTATACGTATGTATATACGTATATCCTTCATC
TTTTCGGAAGTTTCAATGGAAGGATTCCTCTACCAATGCAACACAGTCAATTCCATTTGTT
AGAACAGCTTCCATTGAGTCTCTGCACCTATCCTTTTTTCACCTTCTGGGCCTTTGTTTGTT
TTTGGAAAATAGGATTTGGCTCAGGATTGCCCATTTTTATTAATTCCGGGGTTTCTCTGAAT
TTGAAAGTTCTCACTTAGTAGGTTTCCATACCAAGGCTCAATCCAATTAAGTCCGTAGCGT
CT

- **DQ420000.1 *Triticum monococcum* (Wheat)**

ATTGAGCCTTGGTATGGAAACCTGCTAAGTGGTAACTTCCAAATTCAGAGAAACCCTGGA
ATTAAAAAAGGGCAATCCTGAGCCAAATCCGTGTTTTGAGAAAACAAGGGGTTCTCGAAC
TAGAATACAAAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCG
AGTTAATTACGTTGTGTTGTTAGTGGAATTCCTTCTAGTTCTAAATTAGAGAAAGAGGGGT
TTTATACCTTATACATTTAATAAACACGTATAGATACTGACATAGCAAACGATTAATCACA
GAACTCATATTATATTAGAATTATAATATAGGTTCTTTATCTTTTTTAAAAATGAAATTAG
AAATGATAATGATTATGAAATAAAAAACTCATATCATAATTTTTTTTTTTTCATTATTGGG
AATCCACTCCAATCGAATATTGAATAATCAAATTCTTCAATTAAAATTCAAAGTTTTCGAG
ATCTTTAAAAAAGTGGATTAATCGGACGAGGACAAAGAGAGAGTCCCATTCTACATGTCA
ATACCTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGCCGACTTTATAA

Monolepta signata shared the homology with *O. sativa*, *L. siceraria*, and *T. monococcum*; however, maximum homology was exhibited with *T. monococcum* (Figure 4. 5).

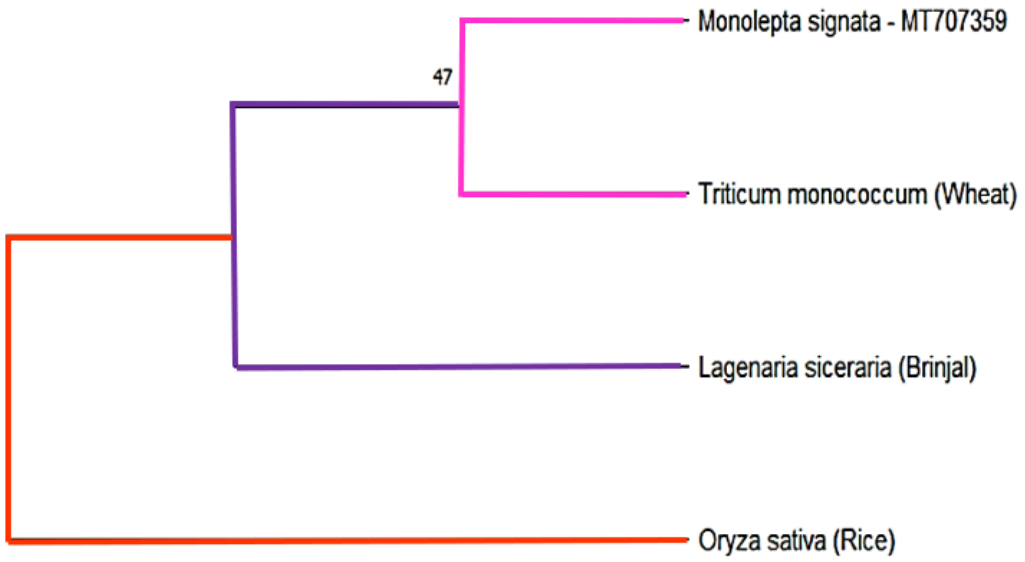

Figure 4. 5: Homology of *Monolepta signata* with its host plants

A genomics analysis AT content of *Monolepta signata* (70.05%) and *T. monococcum* (68.51%) and GC content *of L. fuscipes* (29.95%) and *T. monococcum* (31.49%) was computed with the average of 69.16% and 30.84% respectively.

Further, the closely related pest – host sequences were analyzed for AT, and GC skews, where AT skew was revealed that *M. signata* (-0.10) and *T. monococcum* (0.11) with the average of 0.02, whereas GC skew has resulted that *M. signata* (0.06) and *T. monococcum* (0.09) with the average of 0.08.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *M. signata, T. monococcum* and *L. siceraria*. The nucleotide frequencies for COI were 31.64% (A), 29.92% (T/U), 20.29% (C), and 18.14% (G). The transition/transversion rate ratios were k_1 = 5.957 (purines) and k_2 = 6.603 (pyrimidines). The overall transition/transversion bias was R = 2.972, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Codon-based Test of Neutrality analysis between sequences was performed *by M. signata and T. monococcum* (0.21), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There were 182 positions in the final dataset, and there was nonsignificant ($p<0.05$)

synonymous, and a non- synonymous rate was determined between the species (Table 4. 3).

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 63.08, 28.2and 80.46%, respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 36.92, 36.05and 19.53% respectively percentage was calculated. The pairwise distance among insect-plant species was calculated to be 7.06 for COI (Table 4. 3).

- **B22 *Monolepta signata* - MT707359**

ATAATTGGTGGATTCGGAAATTGGTTAGTTCCATTAATAATTGGAGCTCCTGATATGGCTT
TTCCTCGAATAAATAATATAAGATTTTGATTGTTGCCCCCATCATTATTTTTATTAATTATA
AGAAGAATTGTTGAAAGAGGAGCAGGGACAGGATGAACTGTTTATCCTCCATTATCATCT
AATATTGCTCATGGAGGAGCTTCTGTAGATTTAGCAATTTTTAGACTTCATTTAGCAGGAA
TTTCTTCAATTTTAGGAGCTATTAATTTTATTACAACTATTATTAATATACGTCCAAGAGGA
ATATCTATAGATCGTATACCTTTATTTGTTTGAGCTGTTATAATTACAGCTATTTTACTTCT
ATTATCATTACCAGTATTAGCTGGAGCAATTACAATATTATTAACAGATCGAAACTTAAAT
ACTTC

- **AY792515.1 *Oryza sativa* (Rice)**

GGTATGGAAACCTGCTAAGTGGCAACTTCCAAATTCAGAGAAACCCTGGAATTAAAAAAG
GGGCAATCCTGAGCCAAATCCATGTTTTGAGAAAACAAGCGGTTCTCGAACTAGAACCCA
AAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCGAGTTAATTAC
GTTGTGTTGCTAGCGGAACTCCCTTCTAAATTAGGGAAAGAAGGGCTTTCGAAATCTAATA
CACACGTATAGATACTGGCATAGCAACGATTAATCACAGAACTCATATCATAATATAGGT
TCTTTAATTCTTTTTTAAAATGAAAATAGGAATGATTATGAAATAGAAAATTCATAATTTT
TTTAGAATTGTTGTGAATCCATTCCAATCGAATATTGAGTAATCAAATCCTTCAATTCATA
GTTTTCGAAATCTTTTTAAAAGCGGATTAATCGGACGAGGATAAAGAGAGAGTCCCATTC
TACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGTCGACTTTCTA
AGTCGTGAG

- ***Lagenaria siceraria* (Brinjal)**

TAGCAATTCACATTATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAAT
TTGATTTCTTATCTGTCTACTGTACTGAAACTTCCAAAGTCTTATCCACACAAGGCTTGTGG
TATATATTCTATATGATACACGTACAAACGAACATCCTTGCGCAAGTAATCCCCCTTGTGA
AATTTGAATGATTAACAATAAGCCCTGAAATTTCGTGGATCTTCAAAAAGAAGACTTTGG
AATATCCTCTTATTTACAATTGAGGCCCGTTGGCCTCTTTAATCATTTATCCTTGCATCATA
GACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGGCCGGGATAGCTCAGTT
GGTAGAGCAGAGGACCGTTGGCCTCTTTAATTATTCAAACGAACATCCTTGCGCAAGTAA
TCCCCCTTGTGAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTC
TTATCCAAGCCCTGAAATTTCGTGGATCTTCAAAAATATCCTTTCATTAGCAATTCACATT
ATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAATTTGATTTCTTATCA
CAAGGCTTGTGGTATATATTCTA

- **DQ420000.1 *Triticum monococcum* (Wheat)**

ATTGAGCCTTGGTATGGAAACCTGCTAAGTGGTAACTTCCAAATTCAGAGAAACCCTGGA
ATTAAAAAAGGGCAATCCTGAGCCAAATCCGTGTTTTGAGAAAACAAGGGGTTCTCGAAC

TAGAATACAAAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCG
AGTTAATTACGTTGTGTTGTTAGTGGAATTCCTTCTAGTTCTAAATTAGAGAAAGAGGGGT
TTTATACCTTATACATTTAATAAACACGTATAGATACTGACATAGCAAACGATTAATCACA
GAACTCATATTATATTAGAATTATAATATAGGTTCTTTATCTTTTTTAAAAATGAAATTAG
AAATGATAATGATTATGAAATAAAAAACTCATATCATAATTTTTTTTTTTTCATTATTGGG
AATCCACTCCAATCGAATATTGAATAATCAAATTCTTCAATTAAAATTCAAAGTTTTCGAG
ATCTTTAAAAAAGTGGATTAATCGGACGAGGACAAAGAGAGAGTCCCATTCTACATGTCA
ATACCTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGCCGACTTTATAA

Myllocerus dorsatus, *M. undecimpustulatus*, and *M. viridanus* shared the homology with Zea mays, *Abelmoschus esculentus*, *Ricinus communis* and *Gossypium hirsutum* however *M. dorsatus* was exhibited maximum homology with *A. esculentus* and *M. viridanus* and *M. undecimpustulatus* were shown with *R. communis and G. hirsutum* (Figure 4.6).

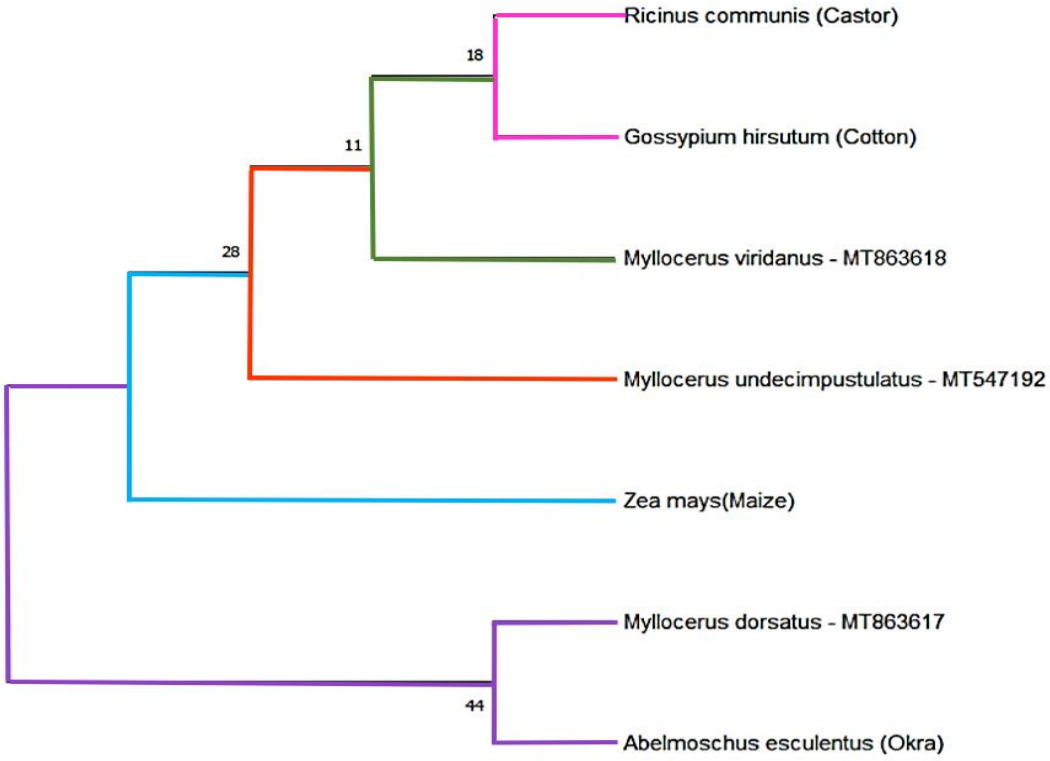

Figure 4. 6: Homology of Myllocerus species with its host plants

A genomics analysis AT content of *M. dorsatus* (65.24%) and *A. esculentus* (61.97%) and GC content of *M. dorsatus* (34.76%) and *A. esculentus* (38.03%) was computed with the average of 62.90% and 37.10% respectively.

Further, the closely related pest – host sequences were analyzed for AT, and GC skews, where AT skew was revealed that *M. dorsatus* (-0.08) and *A. esculentus (*-0.05)

with the average of -0.01 whereas, GC skew resulted that *M. dorsatus* (-0.134) and *A. esculentus (*-0.09) with the average of -0.1.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *M. dorsatus, A. esculentus* and *Z. mays*. The nucleotide frequencies for COI were 28.67% (A), 32.44% (T/U), 17.93% (C), and 20.96% (G). The transition/transversion rate ratios were k_1 = 5.91 (purines) and k_2 = 5.483 (pyrimidines). The overall transition/transversion bias was R = 2.696, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Codon-based Test of Neutrality analysis between sequences was performed for *M. dorsatus and A. esculentus* (0.0005), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There was a total of 407 positions in the final dataset, and there was significant ($p<0.05$) synonymous and non- synonymous rate was determined between the species (Table 4. 3).

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 69.77, 62.7, and 59.61%, respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 30.22, 41.11, and 40.39% respectively percentage was calculated. The pairwise distance among insect-plant species was calculated to be 9.48 for COI (Table 4. 3).

- ***Myllocerus undecimpustulatus* - MT547192**

ATAGTAGGAACCTCCTTAAGAATTTTAATTCGAACAGAACTAGGAAACCCGGGTTCTTTA
ATTGGTGACGATCAAATTTATAACGTAATTGTTACAGCCCATGCTTTCATTATAATTTTCTT
TATAGTTATACCTATAATGATTGGAGGATTTGGGAATTGACTTGTTCCTTTAATATTAGGA
GCACCTGATATAGCTTTTCCTCGTCTTAACAATATAAGATTTTGACTTTTACCCCCATCTCT
CTCCCTTCTTCTTATAAGAAGAATCGTAGATAAGGGGGCAGGTACTGGTTGAACAGTTTAC
CCACCTTTATCAGCTAATATTGCTCATGAAGGATCTTCTGTTGACCTAGCAATTTTTAGACT
CCATATAGCAGGAGTTTCTTCTATCCTAGGAGCCGTAAATTTTATTTCTACAGTAATTAAT
ATACGTCCATCAGGAATATCTTTTGATCGACTACCTCTATTTGTGTGGGCCGTTAAAATTA
CAGCTATTTTACTTCTTTTATCTTTACCAGTTTTAGCTGGAGCTATTACTATACTATTGACA
GATCGTAATATTAATACAT

- ***Myllocerus dorsatus* - MT863617**

TGATGTATTAATATTACGATCAGTTAAAAGTATGGTGATTGCTCCTGCTAATACTGGGAGG
GAAAGAAGAAGAAGAATGGCAGTAATTTTTACAGCTCATACAAATAAAGGAAGACGGTC
AAAAGATATTCCTGAAGGACGTATATTAATAACTGTAGAGATAAAATTAACTGCTCCTAG
GATTGAAGATACCCCAGCTATATTAAGACTAAAAATTGCTAGATCTACAGAAGATCCTTC
ATGGGCGATGTTAGCTGATAAAGGTGGGTAAACCGTTCATCCTGTTCCTGCTCCTTTATCA
ACAATTCTTCTTATTAATAGAAGAGATAATGAAGGGGGTAAAAGTCAGAATCTTATATTA
TTAAGACGTGGAAAAGCTATATCTGGTGCCCCTAATATTAAAGGTACTAGTCAGTTTCCGA

ATCCTCCGATTATTATGGGTATAACTATAAAAAAAATTATAATAAAAGCATGTGCTGTTAC
AATAACGTTGTAAATTTGGTCGTCTCCAATTAGGGATCCTGGGTTTCCTAATTCA

- ***Myllocerus viridanus* - MT863618**

GATAAAGGAGCAGGAACGGGGTGAACAGTCTACCCGCCCCTTTCAGCTAATATCGCCCAT
GAGGGCTCCTCAGTAGATTTAGCAATTTTCAGCCTACATATAGCAGGGGTATCATCAATTC
TAGGTGCAGTAAATTTTATTTCCACAGTAATTAATATACGCCCAATAGGAATATCTTTTGA
TCGACTACCTCTATTTGTATGAGCGGTAAAAATTACAGCAATTCTCCTACTCCTTTCATTAC
CAGTTCTTGCAGGAGCAATTACTATACTTCTCACAGATCGAAATATTAATACATCTTTCTTT
GACCCAGCAGGGGGAG

- **DQ131551.1 *Zea mays* (Maize)**

CGAAATCGGTAGACGCTACGGACTTGATTGTATTGAGCCTTGGTATGGAAACCTGCTAAG
TGGTAACTTCCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATC
CCTTTTTTGAAAAACAAGTGGTTCTCAAACTAGAACCCAAAGGAAAAGGATAGGTGCAGA
GACTCAATGGAAGCTGTTCTAACGAATCGAAGTAATAACGATTAATCACAGAACCCATAT
TATAATATAGGTTCTTTATTTTATTTTTAGAATGAAATTAGGAATGATTATGAAATAGAAA
ATTCATAATTTTTTTTTAGAATTATTGTGAATCTATTCCAATCAAATATTGAGTAATCAAAT
CCTTCAATTCATTGTTTTCGAGATCTTTTAATTTTTAAAAGTGGATTAATCGGACGAGGAT
AAAGAGAGAGTCCCATTCTACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGA
AAATCCGTCGACTTTATAAGTCGTGAGGGTTCAAGTCCCTCTATCCCC

- **KF514666.1 *Abelmoschus esculentus* (Okra)**

CGAAATTGGTAGACGCTGCGGACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAG
TGATAACTTTCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATC
CTATTATTTTACGAAAATAAACATGAACAAAAGTTCAGCAAGCGAGAATAATAATAATAA
AAAAAGGAAAGGATAGGTGCAGAGACTCAATGGAAGCTATTYTAACAAATGGGGTTGAC
TGTTGGTAAAGGAATCCTTATATCGAAATTCCAGAAAGGATGCAAGATATACCTATATAA
AATAAAAAAAAAATAGGTATACTAACGAAAAACTATCTCAAAAAAGACGACCCGAACCC
GTATTTTTTTATATGCAAAATCTATTTATATGAAAAAAAAAGAATTGTTGTGAATCGATTC
CAAGTTGAAGAAAGAATCGAATAGAATATTCATTAATCAAATCATTCACTCCATAGTCTG
ATAAATCTTTTGAAAAACTGATTAATGGACGAGAATAAAGATAGAGTCCCGTTCTACATG
TCAATATCAATACCGACAACAATGAAATTTATAGTAAGA

- **MK797589.1 *Ricinus communis* (Castor)**

ATAACTTTCAATTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAATCCTG
TTTTTCGAAAACAAACAAAGGTTCATAAAGACAGAATAAAAAAAAAAGGATAGGTGCAA
AGACTCAATGGAAGCTGTTCTAACAAACGGAGTTGACTGCGTTGCATTAGTAAAGTAAAG
AAATCCTTCCATCAAAATTCCAGATTCCAGAAAGGATAAAGTAAAGGATAACCATATATA
CATACGGATACGTACTGAAATAATATCTCAAATGATTAATAGGGGGCCGAATCCGTATTTT
TTATCTTTATATGAAAAAAAAAAAAGAATTGTTTTGATTTGAATTGATTCCAAGTTGAAGAA
AGGATTGAATATTAATTGATCACATCATTCACTCCATAGTCTGATAGATAACTGATTAATC
GGACGAGAATAAAGATAGAGTCCCATTCTACATGTCAATATCGACAACAAGGAAATTTAT
AGTAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAA
AACAGGCCCGCTCGACTCCCTTATTATTTTTATCCTATTCTCTCTTTTTGTTAACGGTTCAA
ATTTCGTTATCTTTCTCATTCATTCGATTCTTTCACAAACATATCCGGGCTGGATTTCTTTTC
ACAAGTCTTGGGATAGATATGATATACATAAAAATGAATATCTTTGAGCAAAACAAGAAA
CCCCCATTCCAGTGGAAAATGGAATGATTAACAATCCAAATCATTATTCGAGCTGAAATTT
ACTTTACGAAGTCGTCTTTTTATTCGTTTTTTTAAAAGATACAAAACATTCCGGGTCTGGAT
AAGACTTTAGAATACTTTTTCGTCTTTTTTTTTAATTGACATAGACAAAAGCCGTTTAGTAA
AATGAGGAAGACGACGCGTGAAAATGTC

- **JX874980.1 *Gossypium hirsutum* (Cotton)**

AATTTCATTGTTGTCGGTATTGATATTGACATGTAGAACGGGACTCTATCTTTATTCTCGTC
CGATTAATCAGTTTTTCAAAAGATTTATCAGACTATGGAGTGAATGATTTGATTAATGACT
ATTCTATTCGATTCTTTCTTCAACTTGGAATCGATTCACAACAATTCTTTTTATTTTTCATAT
TTTCATATAAATTGATATTGCATATAATAGAAATAAAAAAAAAATACGGGTTCGGTTCGTCT
TTTTTGAGATAGTTTTTCATTAGTATACCTATAAAAAAAATAGGTATATCTTGCATCCTTTC
TAGAGTTTCGATATTCGATATAAGGATTCCTTTACCAACAGTCAACCCCATTTGTTAGAAT
AGCTTCCATTGAGTCTCTGCACCTATCCTTTCCTTTTTTTCTTATTCTCGCTTGCTGAACCTT
TGTTCATGTTTATTTTCGTAAAATAATAAAATAATAGGATTTGGCTCAGGATTGCCCATTTT
TCATTCCAGGGTTTCTCTGAATTTGAAAGTTATCACTTAGTAGGTTTCCATACCAAGGCTC
AATCCAATT

M. pustulata shared the homology with *Cajanus cajan, Cyamopsis tetragonoloba, S. melongena* and *L. aegyptiaca*, however, maximum homology was exhibited with *Solanum melongena* (Figure 4. 7).

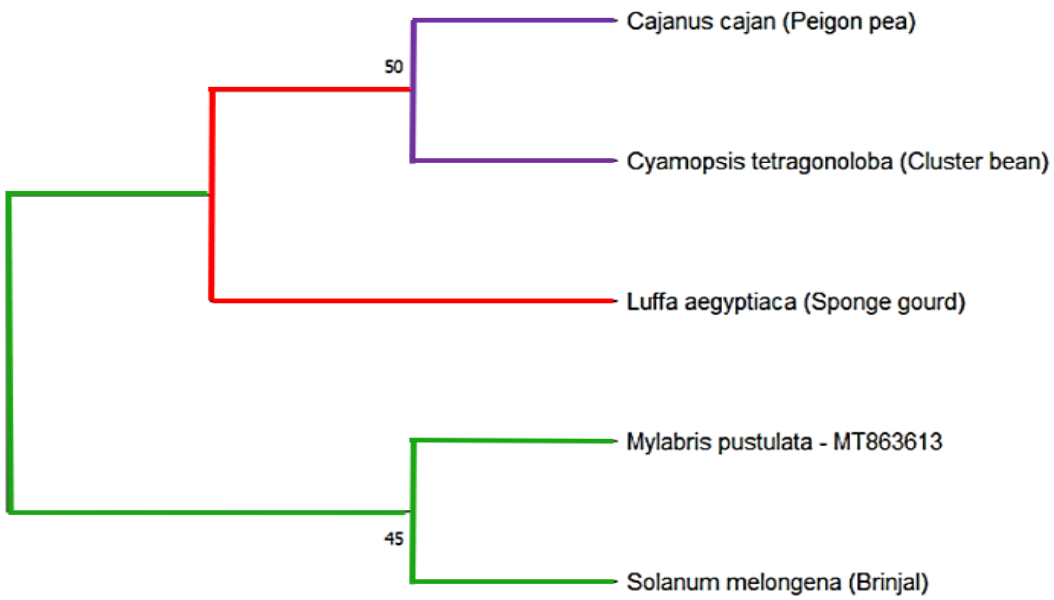

Figure 4. 7: Homology of *Mylabris pustulata* with its host plants

A genomics analysis AT content of *M. pustulata* (60.65%) and *Solanum melongena* (66.81%) and GC content *of M. pustulata* (39.35%) and *Solanum melongena* (33.19%) was computed with the average of 65.58% and 34.42% respectively.

Further, the closely related pest – host sequences were analyzed for AT, and GC skews, where AT skew was revealed that *M. pustulata* (-0.18) and *S. melongena* (0.07) with the average of 0.027, whereas GC skew has resulted that *M. pustulata* (-0.14) and *S. melongena* (0.004) with the average of -0.03.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *M. pustulata, S. melongena* and *L. aegyptiaca*. The nucleotide frequencies for COI were 31.62% (A), 35.69% (T/U), 17.70% (C), and 14.99% (G). The transition/transversion rate ratios were k_1 = 5.841 (purines) and k_2 = 5.192 (pyrimidines). The overall transition/transversion bias was R = 2.431, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Codon-based Test of Neutrality analysis between sequences was performed for *M. pustulata and S. melongena* (0.46), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There were 569 positions in the final dataset, and there was no significant ($p<0.05$) synonymous and non- synonymous rate determined between the species (Table 4. 3).

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 65.40, 63.14and 68.19% respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 34.6, 36.86and 31.80% respectively percentage was calculated. The pairwise distance among insect-plant species was calculated to be 7.85 for COI (Table.4. 3).

- ***Mylabris pustulata* - MT863613**

TACAATGTTATTGTTACAGCCCATGCATTTATCATAATTTTCTTTATGGTAATACCTATCAT
GATTGGCGGCTTTGGGAATTGGCTTGTACCCTTAATGTTAGGGGCCCCTGATATAGCCTTT
CCTCGTATAAACAATATAAGATTTTGATTACTTCCACCTTCATTAACACTTCTAATCATAA
GAAGAATTGTAGAAAATGGTGCAGGAACTGGATGAACGGTGTACCCTCCACTCTCATCTA
ATATTGCCCATGATGGTTCTTCTGTAGATTTAGCCATCTTTAGCCTCCACTTAGCCGGGGTT
TCTTCTATCCTGGGAGCAGTCAATTTCATTTCTACTGTCATCAACATACGCCCAGCTGGAA
TAACATTCGATCGTATACCCTTATTTGTATGAGCAGTTGTTATTACTGCTCTCCTCCTTCTA
TTATCATTACCTGTCCTTGCAGGTGCAATTA

- **EU176155.1 *Solanum melongena***(brinjal)

TAACCTCTGAGCTAAGCGGGCCCACATCACAGAAATCTTATATGCATAGTAATTGACTAA
ACTACTGGAATTGGAATCTTAGTTATTAACTAGTCAATATTATATTGACTATTCTAGAGCA
TAAGGATTAATATAGCGATCTAGAATTTCGATTTATCACAATTCTAATACTAATACACAAT
TCTAATACTAATAATAGTGATTGTAAATATTGTTAATATTCTTTTATTTTCAATTATTTTCA
ATTTGAATTGAATGGTAAATATTATTTTTCATTTCTTTTTTTGGCATTGGAAATACTTTTTAT
TACAGTTCTATATTTGATTCTATATTATATATCTATATCTCTCTCATTCTATATTTAATTTAT
TTCAAATTCTAATTGTTTAATGGAATGGTTAGTTATAACTAATGAGACATTCCTCCGTTTTC
AGGCGAAAGTTAAAAAACAAGAATCGATCGTTCAAGTATTCCAAATTGAATGGCAAAATG
ACAGGAAGCGAGACATATAGATCGGGTATATATCCATCTATATTGAATTGCGGATTCCGA
AATGATAAAATCATTTTTGATTGGACAAAAAAAGGGTCTCCTATAGAAGATAGTTAAGAA
AATCAAAGAGGAGAAAACACGTTTTCGAGATAGGAATCGGTATCTAATGAATTCAATGGT
TCCAGTATAAATGAAAGAAAAAGAAAAAGGAATGACATCCCAACGGGATCCTAATCTCA
AAACAAAAAGAAAGGGGGATATGGCGAAATTGGTAGACGCTACGGACTTAATTGGATTG
AGCCTTGGTATGGAAACTTACTAAGTGATCACTTTCAAATTCAGAGAAACCCTGGAATTA
ACAAAAATGGGCAATCCTGAGCCAAATCCTGTTTTCTCAAAACAAACAAAGGTTCAGAAA

AAAAGGATAGGTGCAGAGACTCAATGGAAGCTATTCTAACAAATGGAGTTAAATGTGTTG
GTAGAGGACTCTTTACATCGAAACTTCAGAAAGAAAAAGAATGAAGTGCAGGAGAAACG
TATATACATACGTATTGAATACTATATCAAATGATTAATGACGACCCGAATCCGTATTTTT
TCTATAAAAAATAGAAGAATTGGTGTGAATCCATTCTACATTGAAGAAAGAATCGAATAT
TCATTGATCAAATCATTCACTCCATAGTCTGATAGATCTTTTGAAGAACTGATTAATCGGA
CGAGAATAAAGATAGAGTCCCGTTCTACATGTCAATACCGGCAACAATGAAATTTATAGT
AAAAGGAAAATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAA
GACTATTTAACTCCCCAACTATTTATCCGACCCCCTTTCCTTAACGGTTCCAAATTCCTTAT
CTTTCTCATCCACTCTATTCTTTTAGAAATGGATTTTTTTTCTAAGAGTAAATGGTTTTCTCT
TATCACAAGCCTTTTGATATCTATGATACACGTAGAAATGAACATCTTTGAGCAAGGAATC
CCTAGTTGAATGATTCCCGATCAATACAATATCATTACTCATACTGAAACTTACAAAATCA
TCTTTTTGAAGATCGAAGAAATTCCCCGGCTTTGAGAAAATTTGTTAATCGACTTACTTGA
CATAGACCCAGTTCTATGATAGAATCAAATAAAATAAGGATACCACCCAAAGGACTCGAA
ATCCTCATGTTAACGGTTCCAATTTCCAATCCAGATTGGTAGGATAGAGGACTGGAAATCC
TCGTTCCAATCTAATCTGGGTTGGAAATCGCCGGGATAGCTCAGTTGGTAGAGCAGAGGA
CTGAAAATCCTCGTG

- **KX268157.1 *Cajanus cajan* (peigon pea)**

GAAACCCTGGAATTCACAATGGGCAATCCTGAGCCAAATCCTGTTTTCCGAAAAGAAATA
AAAGTTTAAAAAGTGATAATAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTGTTCT
AACAAATGGAAAAGATGTGAATCAAATCAATTCAAAGTTGAAGAAAAGATGGAATATTC
ATTGATCAAATCATTCACTCCATCATAATCTGATAGATCCTTTGAAGAACTGATCAATCAG
ACGAGAATAAAGATAGAGTCCTATTCTACATGTCAATACTGACAACAATGAAATTTATAG
TAAGAGGAAAATCCGTCGACTTTAGAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAACC
AAAGGGCCTCTTTAACTTTCAAATTCTTTTTCCTATATCCTCTCTGTCTTTAAGTCGTTATTT
ATGTGTTTTATTCCATTTATTCTTTCACAAAGAAATTGGAATTTTTCTTTTTCTTATCATAAT
TAGGAGTCACAAGTTTTGGAATATATAGAGCATCTTTGAAATATGTAATTATTTGTGTGAA
ACACGTATAATTTTTTTTATGATAATAAAAAAAATGAATATCTTATTTTTGAGCAAGGAAT
CCTCATATGCATGATTAACGATACAAAATAATTACTATTACTGAAACTTACTTATAATAAT
GAAACTTACTTATAATTTTTAATTTTTTGTCTTTTTTTACTTAGTTTACTTAGATTCATTGAC
ATATACTCTAGTAATCTTTTAAAATAAAAGATGAGGCTTATGCGTC

- **AF274360.1 *Cyamopsis tetragonoloba* (Cluster bean)**

CCCTGGAATTCACAATGGGCAATCCTGAGCCAAATCCTATTTTCCAAAAACAAAGAAAAG
TTCAGAAAGCGAGAATAAAAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAC
AAATCAAATGGAGTTGACGACATCTCCTTTCGCGTTAGTAAAGGAAATCCTTCCATCAAA
ATTCCAGAACGGAAAGGATCAAGGATAAGCATGTATATATATACGTATATGTACTGAAAT
ATTATTTCAATTGATCAATGAAGACTCCCAATCTCTATTTGTGAATATTTCTATCACAAATG
AAAGATGTGAATCAAATCAATTCCAAGTTGAAGAAAAAATGGAATATTCATTGATCAAAT
CATTCACTCCATCATAATCTGATAGATCTTTTGAAGAACTGATTAATCAGACGAGAATAAA
GATAGAGTCCCATTCTACATGTCAATACCGACAACAATGAAATTTATAGTAAGAGGAAAA
TCCGTCG

- **KF487460.1 *Luffa aegyptiaca* (Sponge gourd)**

GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAAT
CCTTTTTCCGAAAACAAAAAAAAGGGGTAGGTGCAGAGACTCAATGGAAGCTGTTCTAAC
AAATGGAGTTGACTACGTTGCGTTAGTAAAGGAATGAATCCTTCTATCGAAACTCCAGAA
AAGATGAAAGATAAACGTATTACGTACGGAAATACTATTTGATTAATGACAACCCGAATC
TCTATTTTTTTTTATATATATATAAATATAAAAAATGATATATAAAAATGACCGAATTGTT
ATGAATCGATTCCAATATCTCCAAGTTGAAAAAAGAATCGAATATTCATTGATCAAATCAT
TTACTCCATCATACATAGTCTGATAGATCTTTTGAAGAACTGATTGATCARATGCGAATAG
AATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAAAAATGAAATTTATAGTAAGA

GGAAAATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAACCCTA
AAAAGGCCCGTTGGCCTCTTTAATTATTTATTATCCTTTCATTAGCAATTCACAATTCGTTA
TGTTTCTCATTCATTCTACTCTTTCACAAGCGTATCTGAGCGGAAATTTGATTTCTTAGCAC
AAGACTTGTGGTATATATTTATATGATACACGTACAAACGAACATCCTTGCGCAAGGAAT
CCCCGTTGTTAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTCT
TATCCAAGCCCTGAAATTTCGTGGATCTTTAAAAAGAAGACTTTGGAATACCTTTTTTCTT
ATTTACAATTGACATAGACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGG
CCGGGATAGCTCAGTTGGTAGAGCANAGGACTGAAAATC

The phylogeny analysis for ***Oxycetonia versicolor*** revealed shared the homology with *L. aegyptiaca*, *Vigna unguiculate*, *Abelmoschus esculentus*, and *Lagenaria siceraria* and; the maximum homology was exhibited with *L. aegyptaca* (Figure 4.8)

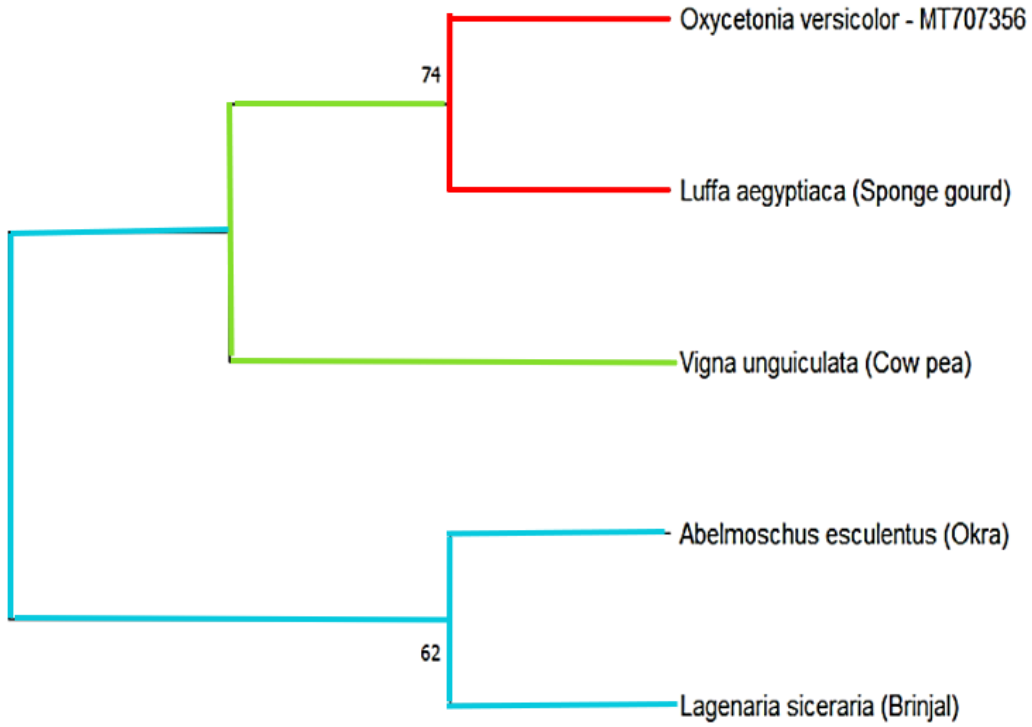

Figure 4. 8: Homology of *O. versicolor* with its host plants

The analysis of bases was computed where AT content of *O. versicolor* (66.95%) and *L. aegyptaca* (70%) and GC content of *O. versicolor* (33.05%) and *L. aegyptaca* (30%) was found with an average of 68.51% and 31.48%, respectively.

Further, the closely related pest – host sequences were analyzed for, and GC skews, where AT skew analysis was revealed that *O. versicolor* (-0.15) and *L. aegyptaca (*-0.10) with an average of -0.12, whereas the analysis of GC skew resulted that *O. versicolor (-0.05*) and *L. aegyptaca (0.09*) with an average of 0.02.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *O. versicolor, L. aegyptaca* and *V. unguiculate.* The nucleotide frequencies for COI were 29.12% (A), 36.56% (T/U), 18.51% (C), and 15.81% (G). The transition/transversion rate ratios are k_1 = 5.007 (purines) and k_2 = 5.187 (pyrimidines). The overall transition/transversion bias was found to be R = 2.351, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$.

The neutrality analysis based on codon between sequences was performed for *O. versicolor* and *L.aegyptaca* (0.088), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There were 152 positions in the final dataset and was nonsignificant ($p<0.05$) for the synonymous and non- synonymous rate determined between the species (Table 4.3)

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 64.31, 60.80and, 80.5% respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 35.69, 39.2and 19.50% respectively percentage was calculated. The pairwise distance among insect-plant species was calculated to be 2.79 for COI (Table 4.3).

- ***Oxycetonia versicolor* - MT707356**

ATAATTTTTTTCATAGTAATACCAATTATGATTGGTGGTTTTGGAAATTGGCTTGTACCCTT
AATACTAGGGGCCCCTGATATAGCCTTTCCTCGAATAAATAACATAAGATTTTGATTATTG
CCTCCATCATTAACTTTACTTTTAATAAGAAGAATAGTAGAAAGAGGGGCAGGAACTGGA
TGAACTGTCTATCCGCCTCTTTCAAGAAATATTGCTCATAGAGGAGCATCTGTTGATTTAG
CTATTTTTAGACTTCATCTTGCAGGTATTTCATCAATTTTAGGTGCTGTTAATTTTATTACT
ACTGTAATTAACATACGATCTGCAGGAATAACTTTTGATCGAATACCTTTATTTGTTTGAT
CTGTTGCTCTTACTGCCTTATTACTTTTATTATCCTTACCTGTCTTAGCAGGAGCTATTACA
ATACTACTTACAGATCGAAATATTAATACTTCCTTCTTTGACC

- **KF487460.1 *Luffa aegyptiaca* (Sponge gourd)**

GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAAT
CCTTTTTCCGAAAACAAAAAAAAGGGGTAGGTGCAGAGACTCAATGGAAGCTGTTCTAAC
AAATGGAGTTGACTACGTTGCGTTAGTAAAGGAATGAATCCTTCTATCGAAACTCCAGAA
AAGATGAAAGATAAACGTATTACGTACGGAAATACTATTTGATTAATGACAACCCGAATC
TCTATTTTTTTTTATATATATATAAATATAAAAAATGATATATAAAAATGACCGAATTGTT
ATGAATCGATTCCAATATCTCCAAGTTGAAAAAAGAATCGAATATTCATTGATCAAATCAT
TTACTCCATCATACATAGTCTGATAGATCTTTTGAAGAACTGATTGATCARATGCGAATAG
AATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAAAAATGAAATTTATAGTAAGA
GGAAAATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAACCCTA
AAAAGGCCCGTTGGCCTCTTTAATTATTTATTATCCTTTCATTAGCAATTCACAATTCGTTA
TGTTTCTCATTCATTCTACTCTTTCACAAGCGTATCTGAGCGGAAATTTGATTTCTTAGCAC
AAGACTTGTGGTATATATTTATATGATACACGTACAAACGAACATCCTTGCGCAAGGAAT
CCCCGTTGTTAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTCT
TATCCAAGCCCTGAAATTTCGTGGATCTTTAAAAAGAAGACTTTGGAATACCTTTTTTCTT

ATTTACAATTGACATAGACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGG
CCGGGATAGCTCAGTTGGTAGAGCANAGGACTGAAAATC

- **MK883492.1 *Vigna unguiculata* (Cow pea)**

ACTTAATTGGATTGAGTTTTGGTATGGAAACTTACCAAGTAATAATTTTCAAATTCAGAGA
AACCCTGGAATTCACAATGGGCAATCCTGAGCCAAATCCTGTTTTCTGAAAACAAATAAA
AATTCAGAAAGTGATAATAAAAAAGGGATAGGTGCAGAGACTCTATGGAAGCTGTTCTAA
CAAACGAAATTGACGACTTTTTTTATTGCATTAGTAAAAGAATCCTTTCACCAAAATTACA
GGAATGGATCAAAGATAAACATTTAGAAACATATATATATACTGAAATACTCTTTCAATTT
GTTACTTTTTATTTTATTATTGAAGATCTATTTGTGATAAAAATATTCACAAATGAAAAAT
GTGAATCAAATCAATTCGAAGTTGAAGAAAAGATGGAATATTTATTGATGAAATTATTCA
CTTCATCATAATCGGCTAAAACCCTTGAAGAACTGATAAATCAGATGAGAATAAAGATAG
AGTCCTATTCTACATGTCAATACCGACAACAATGAAATTGAAAGTAAGAGGAAAATCCGT
CGACTTAAGAAATCATGAGG

- **KF514666.1 *Abelmoschus esculentus* (Okra)**

CGAAATTGGTAGACGCTGCGGACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAG
TGATAACTTTCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATC
CTATTATTTTACGAAAATAAACATGAACAAAAGTTCAGCAAGCGAGAATAATAATAATAA
AAAAAGGAAAGGATAGGTGCAGAGACTCAATGGAAGCTATTYTAACAAATGGGGTTGAC
TGTTGGTAAAGGAATCCTTATATCGAAATTCCAGAAAGGATGCAAGATATACCTATATAA
AATAAAAAAAAAAATAGGTATACTAACGAAAAACTATCTCAAAAAAGACGACCCGAACCC
GTATTTTTTTATATGCAAAATCTATTTATATGAAAAAAAAAGAATTGTTGTGAATCGATTC
CAAGTTGAAGAAAGAATCGAATAGAATATTCATTAATCAAATCATTCACTCCATAGTCTG
ATAAATCTTTTGAAAAACTGATTAATGGACGAGAATAAAGATAGAGTCCCGTTCTACATG
TCAATATCAATACCGACAACAATGAAATTTATAGTAAGA

- ***Lagenaria siceraria* (Brinjal)**

TAGCAATTCACATTATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAAT
TTGATTTCTTATCTGTCTACTGTACTGAAACTTCCAAAGTCTTATCCACACAAGGCTTGTGG
TATATATTCTATATGATACACGTACAAACGAACATCCTTGCGCAAGTAATCCCCCTTGTGA
AATTTGAATGATTAACAATAAGCCCTGAAATTTCGTGGATCTTCAAAAAGAAGACTTTGG
AATATCCTCTTATTTACAATTGAGGCCCGTTGGCCTCTTTAATCATTTATCCTTGCATCATA
GACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGGCCGGGATAGCTCAGTT
GGTAGAGCAGAGGACCGTTGGCCTCTTTAATTATTCAAACGAACATCCTTGCGCAAGTAA
TCCCCCTTGTGAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTC
TTATCCAAGCCCTGAAATTTCGTGGATCTTCAAAAATATCCTTTCATTAGCAATTCACATT
ATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAATTTGATTTCTTATCA
CAAGGCTTGTGGTATATATTCTA

Protaetia aurichalcea shared the homology with *L. aegyptiaca, L. siceraria, V. unguiculate,* and *A. esculentus*; however, maximum homology was exhibited with *L. siceraria* (Figure 4. 9)

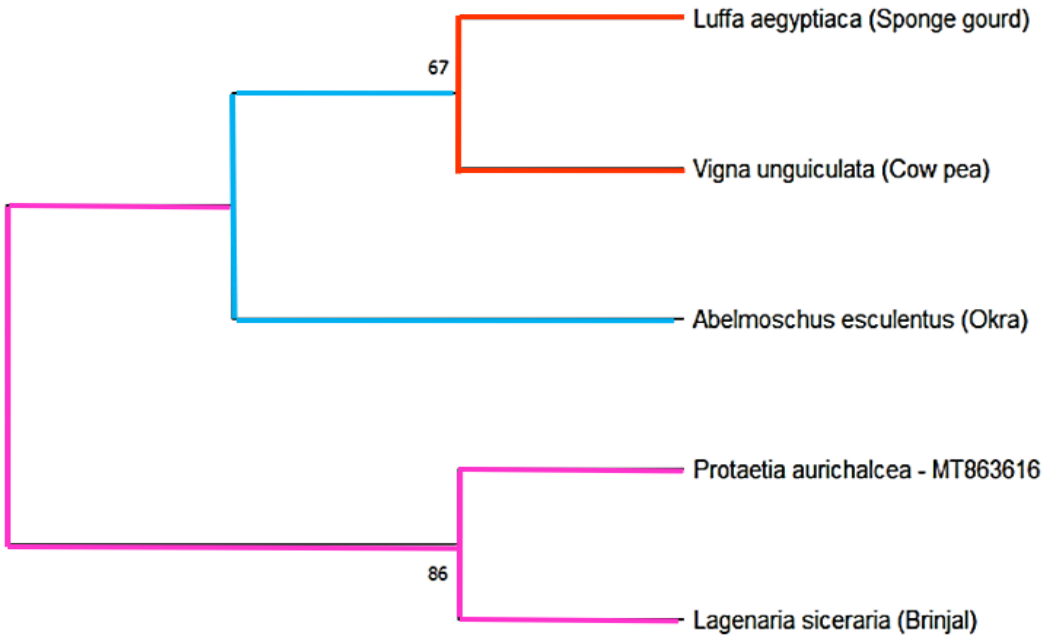

Figure 4. 9: Homology of *Protaetia aurichalcea* with its host plants

A genomics analysis AT content of *P. aurichalcea* (64.77%) and *L. siceraria* (63.72%) and GC content of *P. aurichalcea* (35.23%) and *L. siceraria* (36.28%) was computed with the average of 64.21% and 35.79% respectively.

Further, the closely related pest – host sequences were analyzed for AT and GC skews, where AT skew was revealed that *P. aurichalcea* (-0.09) and *L. siceraria (*-0.07) with the average of -0.08, whereas GC skew has resulted that *P. aurichalcea* (-0.15) and *L. siceraria* (-0.11) with the average of -0.13.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *P. aurichalcea, L. siceraria* and *A, esculentus*. The nucleotide frequencies for COI were 34.09% (A), 31.30% (T/U), 18.43% (C), and 16.17% (G). The transition/transversion rate ratios were k_1 = 5.397 (purines) and k_2 = 4.665 (pyrimidines). The overall transition/transversion bias was R = 2.267, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Codon-based Test of Neutrality analysis between sequences was performed *by P. aurichalcea and L. siceraria* (0.15), where the analysis involved two closer pest-host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There was a total of 199 positions in the final dataset, and there was nonsignificant

($p<0.05$) synonymous, and a non- synonymous rate was determined between the species (Table 4.3)

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 57.87, 60.31and 74.49%, respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 42.13, 39.69and 25. 51%, respectively, the percentage was calculated. The pairwise distance among insect-plant species was calculated to be 5.82 for COI (Table 4.3).

- ***Protaetia aurichalcea* - MT863616**

ATCCGAGCCGAACTAGGAAACCCTGGATCTTTAATTGGCGACGACCAAATTTATAATGTA
ATTGTTACAGCTCACGCTTTCATCATAATTTTTTTCATAGTAATACCAATCATAATTGGTGG
ATTTGGAAATTGACTTGTCCCTCTAATACTTGGGGCACCAGATATAGCTTTCCCACGAATA
AACAACATAAGTTTTTGACTTCTCCCCCCTTCACTAACTTTACTTCTAATAAGAAGAATAG
TTGAAAGAGGAGCAGGAACTGGATGAACTGTTTATCCCCCCTTATCTAGAAATATTGCTCA
TAGTGGAGCTTCAGTAGACCTAGCTATTTTCAGATTACATCTAGCTGGAATTTCCTCAATT
TTAGGTGCTGTTAATTTTATTACTACTGTAATTAATATACGATCAACAGGAATAACATTCG
ATCGAATACCTTTATTTGTTTGATCTGTTGCTTTAACTGCTTTACTTCTTCTACTTTCACTTC
CTGTCTTAGCTGGAGCAATTACTATACTTTTAACTGATCGAAATATTAATACCTC

- **KF487460.1 *Luffa aegyptiaca* (Sponge gourd)**

GTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAAAAATGGGCAATCCTGAGCCAAAT
CCTTTTTCCGAAAACAAAAAAAAGGGGTAGGTGCAGAGACTCAATGGAAGCTGTTCTAAC
AAATGGAGTTGACTACGTTGCGTTAGTAAAGGAATGAATCCTTCTATCGAAACTCCAGAA
AAGATGAAAGATAAACGTATTACGTACGGAAATACTATTTGATTAATGACAACCCGAATC
TCTATTTTTTTTTATATATATATAAATATAAAAAATGATATATAAAAATGACCGAATTGTT
ATGAATCGATTCCAATATCTCCAAGTTGAAAAAAGAATCGAATATTCATTGATCAAATCAT
TTACTCCATCATACATAGTCTGATAGATCTTTTGAAGAACTGATTGATCARATGCGAATAG
AATAAAGATAGAGTCCCATTCTACATGTCAATACCGACAAAAATGAAATTTATAGTAAGA
GGAAAATCCGTCGACTTTAAAAATCGTGAGGGTTCAAGTCCCTCTATCCCCAAAACCCTA
AAAAGGCCCGTTGGCCTCTTTAATTATTTATTATCCTTTCATTAGCAATTCACAATTCGTTA
TGTTTCTCATTCATTCTACTCTTTCACAAGCGTATCTGAGCGGAAATTTGATTTCTTAGCAC
AAGACTTGTGGTATATATTTATATGATACACGTACAAACGAACATCCTTGCGCAAGGAAT
CCCCGTTGTTAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTCT
TATCCAAGCCCTGAAATTTCGTGGATCTTTAAAAAGAAGACTTTGGAATACCTTTTTTCTT
ATTTACAATTGACATAGACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGG
CCGGGATAGCTCAGTTGGTAGAGCANAGGACTGAAAATC

- ***Lagenaria siceraria* (Brijal)**

TAGCAATTCACATTATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAAT
TTGATTTCTTATCTGTCTACTGTACTGAAACTTCCAAAGTCTTATCCACACAAGGCTTGTGG
TATATATTCTATATGATACACGTACAAACGAACATCCTTGCGCAAGTAATCCCCCTTGTGA
AATTTGAATGATTAACAATAAGCCCTGAAATTTCGTGGATCTTCAAAAAGAAGACTTTGG
AATATCCTCTTATTTACAATTGAGGCCCGTTGGCCTCTTTAATCATTTATCCTTGCATCATA
GACCAAAGTCATCTATTAAAATAAGGATAATGTGTCGGAAATGGCCGGGATAGCTCAGTT
GGTAGAGCAGAGGACCGTTGGCCTCTTTAATTATTCAAACGAACATCCTTGCGCAAGTAA
TCCCCCTTGTGAAATTTGAATGATTAACAATACTGTCTACTGTACTGAAACTTCCAAAGTC
TTATCCAAGCCCTGAAATTTCGTGGATCTTCAAAAATATCCTTTCATTAGCAATTCACATT

ATGTTTCTCATTCATTCGACTCTTTCACAAGCGTATTTGAGTGGAAATTTGATTTCTTATCA
CAAGGCTTGTGGTATATATTCTA

- **MK883492.1 *Vigna unguiculata* (Cow pea)**

ACTTAATTGGATTGAGTTTTGGTATGGAAACTTACCAAGTAATAATTTTCAAATTCAGAGA
AACCCTGGAATTCACAATGGGCAATCCTGAGCCAAATCCTGTTTTCTGAAAACAAATAAA
AATTCAGAAAGTGATAATAAAAAAGGGATAGGTGCAGAGACTCTATGGAAGCTGTTCTAA
CAAACGAAATTGACGACTTTTTTTATTGCATTAGTAAAAGAATCCTTTCACCAAAATTACA
GGAATGGATCAAAGATAAACATTTAGAAACATATATATATACTGAAATACTCTTTCAATTT
GTTACTTTTTATTTTATTATTGAAGATCTATTTGTGATAAAAATATTCACAAATGAAAAAT
GTGAATCAAATCAATTCGAAGTTGAAGAAAAGATGGAATATTTATTGATGAAATTATTCA
CTTCATCATAATCGGCTAAAACCCTTGAAGAACTGATAAATCAGATGAGAATAAAGATAG
AGTCCTATTCTACATGTCAATACCGACAACAATGAAATTGAAAGTAAGAGGAAAATCCGT
CGACTTAAGAAATCATGAGG

- **KF514666.1 *Abelmoschus esculentus* (Okra)**

CGAAATTGGTAGACGCTGCGGACTTAATTGGATTGAGCCTTGGTATGGAAACCTACTAAG
TGATAACTTTCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATC
CTATTATTTTACGAAAATAAACATGAACAAAAGTTCAGCAAGCGAGAATAATAATAATAA
AAAAAGGAAAGGATAGGTGCAGAGACTCAATGGAAGCTATTYTAACAAATGGGGTTGAC
TGTTGGTAAAGGAATCCTTATATCGAAATTCCAGAAAGGATGCAAGATATACCTATATAA
AATAAAAAAAAAAATAGGTATACTAACGAAAAACTATCTCAAAAAAGACGACCCGAACCC
GTATTTTTTTATATGCAAAATCTATTTATATGAAAAAAAAAGAATTGTTGTGAATCGATTC
CAAGTTGAAGAAAGAATCGAATAGAATATTCATTAATCAAATCATTCACTCCATAGTCTG
ATAAATCTTTTGAAAAACTGATTAATGGACGAGAATAAAGATAGAGTCCCGTTCTACATG
TCAATATCAATACCGACAACAATGAAATTTATAGTAAGA

Sitophilus oryzae shared the homology with *Z. mays*, *T. monococcum*, *Pennisetum glaucum, and Oryza sativa*; however, maximum homology was exhibited *Z. mays* (Figure 4. 10).

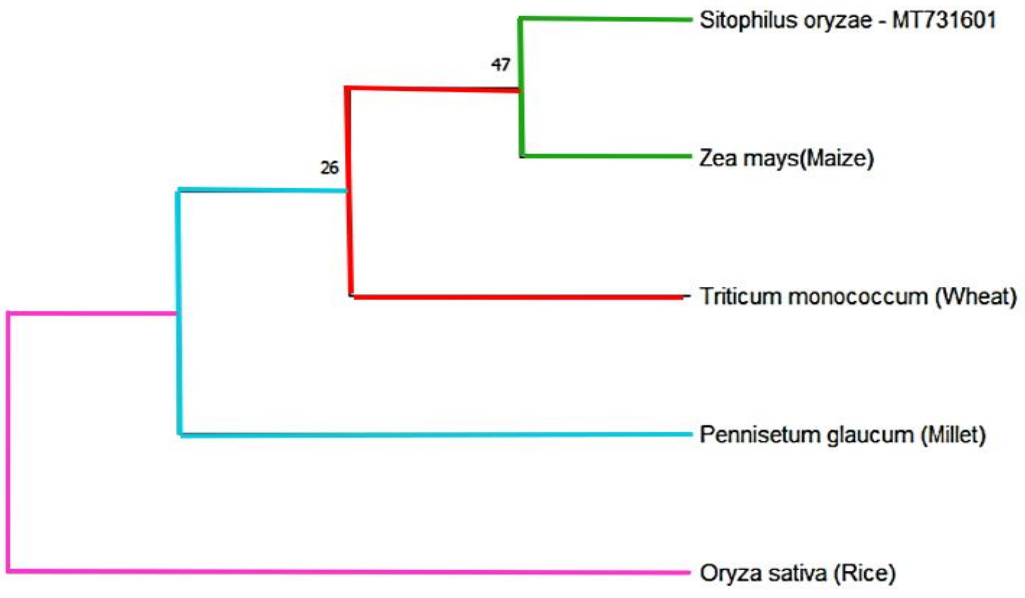

Figure 4. 10: Homology of *Sitophilus oryzae* with its host plants

A genomics analysis AT content of *S. oryzae* (63.86%) and *Z. mays* (65.98%) and GC content of *S. oryzae* (36.14%) and *Z. mays* (34.02%) was found to be the average of 65.18% and 34.81%, respectively.

Further, the closely related pest – host sequences were analyzed for GC and AT skews, where GC skew has resulted that *S. oryzae* (-0.16) and *Z. mays* (0.06) with the average of -0.02 whereas AT skew was revealed that *S. oryzae* (-0.004) and *Z. mays* (0.07) with the average of 0.04.

Maximum Composite Likelihood estimate of the pattern of nucleotide substitution analysis was carried out with *S. oryzae*, *Z. mays* and *T. monococcum*. The nucleotide frequencies for COI were 35.44% (A), 30.63% (T/U), 16.52% (C), and 17.42% (G). The transition/transversion rate ratios are k_1 = 6.47 (purines) and k_2 = 5.745 (pyrimidines). The overall transition/transversion bias was R = 2.769, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Codon-based Test of Neutrality analysis between sequences was performed for *S. oryzae and Z. mays* (0.06), where the analysis involved two closer pest - host nucleotide sequences. It eliminated all unclear positions for each pair of sequences. There were 157 positions in the final dataset, and there was no significant ($p<0.05$) synonymous and non- synonymous rate determined between the species (Table 4. 3).

Similarly, the A+T contents of the 1st, 2nd, and 3rd codons were 61.05, 70.42and 64.08%, respectively, and G+C contents of the 1st, 2nd, and 3rd codons were 38.95, 29. 58and 35.92%, respectively, the percentage was calculated. The pairwise distance among insect-plant species was calculated to be 4.49 for COI (Table 4.3).

- ***Sitophilus oryzae* - MT731601**

TACCAATTATAATTGGAGGATTTGGAAACTGATTAATCCCATTAATATTAGGAGCCCCAGA
TATAGCATTCCCCCGTTTAAATAATATAAGATTTTGATTACTTCCACCCTCCTTAACTCTTT
TACTAATAAGAAGATTTATTGAAAAGGGAGCAGGAACAGGATGAACCGTCTACCCCCCGC
TCTCATCCAATATTGCCCATGAAGGAGCTTCTGTTGATCTGGCCATTTTCAGTTTACATATA
GCAGGAATTTCATCTATTCTAGGAGCTATTAATTTTATTACAACAGCCTATAATATACGAC
CCTCAGGAATATTAT

- **AY792515.1 *Oryza sativa* (Rice)**

GGTATGGAAACCTGCTAAGTGGCAACTTCCAAATTCAGAGAAACCCTGGAATTAAAAAAG
GGGCAATCCTGAGCCAAATCCATGTTTTGAGAAAACAAGCGGTTCTCGAACTAGAACCCA

AAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCGAGTTAATTAC
GTTGTGTTGCTAGCGGAACTCCCTTCTAAATTAGGGAAAGAAGGGCTTTCGAAATCTAATA
CACACGTATAGATACTGGCATAGCAACGATTAATCACAGAACTCATATCATAATATAGGT
TCTTTAATTCTTTTTTAAAATGAAAATAGGAATGATTATGAAATAGAAAATTCATAATTTT
TTTAGAATTGTTGTGAATCCATTCCAATCGAATATTGAGTAATCAAATCCTTCAATTCATA
GTTTTCGAAATCTTTTTAAAAGCGGATTAATCGGACGAGGATAAAGAGAGAGTCCCATTC
TACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGTCGACTTTCTA
AGTCGTGAG

- **EU939991.1 *Pennisetum glaucum* (Millet)**

GATTGTATTGAGCCTTGGTATGGAAACCTGCTAAGTGATAACTTCCAAATTCAGAGAAAC
CCTGGAATGAAAAATGGGCAATCCTGAGCCAAATCCCTTTTTTGAAAAAACAAGTGGTTC
TAAAACTGGAACCCAAAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAAC
GAATCGAAGTAATTACGTTGTGTTGGTAGGGGAACTCCCTCGAAATACTAGAAAGAAGGG
CTTTATACATTTAATACACACGTATAGATACTGACATAGTAAACGATTAATCACAGAACCC
CTATCATAATATAGGTTCTTTATTTTATATTTTTTTCTAGAATGAAATTAGGAATGATTATG
AAATAGAAAATTCTGAATTTTTTTAGAATTATTGTGAATCCATTCCAATCGAATATTGAGT
AATCAAATCCTTCAATTCATTGTTTTGAGATCAAAAGTGGATTAATCGAACGAGGATAAA
GAGAGAGTCCCATTCTACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGAAAA
TCCGTCGACTTTATAAGTCGTGAGGGTTCAAGTCCCTCTATCCCCACCCAAACCCTCTTTTA
TTCCCTGACCATAGTAGTTATCCTTTTTTTCTTTTATCAATGGGTTTAAGATTCATTAGCTTT
CTCATTCGACTCTTTCTTTCACAAAGGAGTGCGATGAGAACTCAATGAATCTTATGCTATT
CATTAAATAGAATATTTCTTTTTTATTTGATAGGACTACCCCCCCCCCGCTCCATTCCAAAT
TTGGAATGGAATACTTTATNGATTTTTTAGTCCCGTTAATTGACATAGATGCAAATACTCT
ACTAGGATGATGCNCAANAAA

- **DQ131551.1 *Zea mays* (Maize)**

CGAAATCGGTAGACGCTACGGACTTGATTGTATTGAGCCTTGGTATGGAAACCTGCTAAG
TGGTAACTTCCAAATTCAGAGAAACCCTGGAATGAAAAATGGGCAATCCTGAGCCAAATC
CCTTTTTTGAAAAACAAGTGGTTCTCAAACTAGAACCCAAAGGAAAAGGATAGGTGCAGA
GACTCAATGGAAGCTGTTCTAACGAATCGAAGTAATAACGATTAATCACAGAACCCATAT
TATAATATAGGTTCTTTATTTTATTTTTAGAATGAAATTAGGAATGATTATGAAATAGAAA
ATTCATAATTTTTTTTTAGAATTATTGTGAATCTATTCCAATCAAATATTGAGTAATCAAAT
CCTTCAATTCATTGTTTTCGAGATCTTTTAATTTTTAAAAGTGGATTAATCGGACGAGGAT
AAAGAGAGAGTCCCATTCTACATGTCAATACTGACAACAATGAAATTTCTAGTAAAAGGA
AAATCCGTCGACTTTATAAGTCGTGAGGGTTCAAGTCCCTCTATCCCC

- **DQ420000.1 *Triticum monococcum* (Wheat)**

ATTGAGCCTTGGTATGGAAACCTGCTAAGTGGTAACTTCCAAATTCAGAGAAACCCTGGA
ATTAAAAAAGGGCAATCCTGAGCCAAATCCGTGTTTTGAGAAAACAAGGGGTTCTCGAAC
TAGAATACAAAGGAAAAGGATAGGTGCAGAGACTCAATGGAAGCTGTTCTAACGAATCG
AGTTAATTACGTTGTGTTGTTAGTGGAATTCCTTCTAGTTCTAAATTAGAGAAAGAGGGGT
TTTATACCTTATACATTTAATAAACACGTATAGATACTGACATAGCAAACGATTAATCACA
GAACTCATATTATATTAGAATTATAATATAGGTTCTTTATCTTTTTTAAAAATGAAATTAG
AAATGATAATGATTATGAAATAAAAAACTCATATCATAATTTTTTTTTTTTCATTATTGGG
AATCCACTCCAATCGAATATTGAATAATCAAATTCTTCAATTAAAATTCAAAGTTTTCGAG
ATCTTTAAAAAAGTGGATTAATCGGACGAGGACAAAGAGAGAGTCCCATTCTACATGTCA
ATACCTGACAACAATGAAATTTCTAGTAAAAGGAAAATCCGCCGACTTTATAA

4. 4 Discussion

The study of insect-plant interactions is at the center of a vibrant group of scientists encircling a broad range of biological questions from molecular to ecosystem level, all united by evolutionary biology. Innovations and computational methods have recently revolutionized this research area, including the next-generation sequencing and gene-editing technology (Giron *et al.*, 2018). Molecular genetics, genomics, chemistry, neuroscience, behavioural research, and other methods have allowed us to get a more detailed understanding of complex biological networks, the physiological, economic, and evolutionary mechanisms of these relationships, and the genetic basis of traits, and to challenge previously unanswerable hypotheses. Plants communicate with multiple biotic partners ranging from parasites to mutualists; plants enable the signaling networks in response to parasites, herbivores, and beneficial organisms which eventually overlap (Endara *et al.*, 2017), providing insects novel adaptive capacities, enabling the ability to expand to new ecological niches (Fordyce, 2010; Jurado-Rivera *et al.*, 2009).

The diversity of insect-plant interaction remains a significant challenge for understanding species richness and its ecological functioning. However, the difficulty in the establishment of host associations hampers the study of plant-herbivore interactions and their role in promoting species richness (Lewinsohn and Roslin, 2008). The proof of DNA-based plant identification from insect tissue (Jurado-Rivera *et al.*, 2009). now provides an innovative method with great potential for studying the most complex plant-insect interaction on earth and has resolved the long-standing questions about the factors promoting species diversity. The conceptual framework for the study of plant-herbivore interaction was constructed based on phylogenetic patterns among host plants and phytophagous insects, butterflies of the superfamily Papilionoideae and their host plants (Ferrer-Paris *et al.*, 2013). Precise identification of the plant host is influenced by the reference DNA database's broadness and the discriminatory power of the locus used for sequencing. In the present study, the trnL intron was selected as it is a potential barcoding marker, it is highly conserved, and it has the highest level of coverage in GenBank (Navarro *et al.*, 2010; Taberlet *et al.*, 2007). Further, trnL primers are universal and possess the power in phylogenetic analysis across various hierarchical (Bremer *et al.*, 2002; Navarro *et al.*, 2010; Shaw *et al.*, 2007). In line with this, the

present study was aimed to decipher the interaction of 16 coleopteran species and their host plant. For all the insects, homology modelling using maximum parsimony was carried out, and genomic comparisons were made using trnl and COI sequence. Based on the fields' observations and the infestation (Chapter II), plants and their coleopteran pest were selected. Hence, different plant species with a single/common pest were compared. The homology modelling analysis revealed that the trnL intron sequences with COI were sufficient to discriminate among all individuals in the agriculture fields. The results obtained are thus in accordance with the work of Navarro *et al.,* 2010 demonstrated that the trnL intron is a powerful phylogenetic marker for reconstructing host plant lineages' relationships.

All the Coleopteran pests selected were on a broad range of plant families: Malvaceae, Solanaceae, Poaceae, Moringaceae, Malvaceae, Apiaceae, Cucurbitaceae, and Fabaceae, which was showing a high trophic diversity across significant groups of angiosperms. While some host sequences were closely related and might indeed be from the same host family, the sequence variation still indicates host plants' diversity. Moreover, the host records may be highly variable for a species when more than one individual was available for analyses (e.g *Aulacophora foveicollis sharing homology with Moringa oleifera*.), but host conservation derived from high sequence similarity is typically found (e.g. *Oxycetonia versicolor with Luffa aegyptiaca*). Furthermore, host specificity was not phylogenetically conserved in the studied group of coleopteran, as sister taxa usually feed on different plant families and generally very distant ones phylogenetically, and reciprocally with similar host plants being used by a divergent phytophagous pest (Novotny *et al.,* 2002; Novotny and Weiblen, 2005; Barone, 2002)

The present study also demonstrated that the trnL and COI locus discriminated between congeneric based on several nucleotide changes; for instance, *Altica cyanea* was found to be closed to *Lagenaria siceraria; Aulacophora indica with Lagenaria siceraria; Aulacophora foveicollis with Moringa oleifera; Chiloba orientalis with Oryza sativa; Lanelater fuscipes* with *Daucus carota*; *Monolepta signata with Triticum monococcum; Mylabris pustulata* with *Solanum melongena; Myllocerus dorsatus with Abelmoschus esculentus; Myllocerus viridanus with Gossypium hirsutum; Myllocerus undecimpustulatus with Zea mays;Oxycetonia versicolor* with *Luffa aegyptiaca; Protaecia aurichalcea with Lagenaria siceraria* and *Sitophilus oryzae with Zea mays.*

Similarly, the studies conducted by García-Robledo *et al.*, (2013) in decoding the host plants to the genus level was higher than that in other reported studies (Navarro *et al.*, 2010; Jurado-Rivera *et al.*, 2009) due to usage of more than one molecular marker. Therefore, the present study results are consistent with the findings for leaf rolling beetles (García-Robledo *et al.,* 2013) which also proved that more than one marker should be used while reconstructing a herbivore network plant interactions.

Further, to analyze the ambiguity of bases, pairwise distance, AT /GC content, non-synonymous and synonymous mutation rate were analyzed between the coleopteran pest and host plant. Among all the Coleopterans and host plant analyzed, it was found that AT biasness was more in all the Coleoptera species. Similarly, the Transition/ transversion ratio was found to be in the range of 2.19 to 3.86, suggesting a high transition rate among the species and host plant, which is more favored in the evolution (Stoltzfus and Norris, 2015). This intraspecific variation for the locus is not unique and has been studied previously (Taberlet *et al.,* 2007; Tsai *et al.,* 2008). However, it does mean that in a sequence of 1-2bp differences in *trnL* sequences, the association with intraspecific or interspecific variation without being able to identify the plants in another way remains elusive. While, levels of sequence divergence in *COI* near the species level were much higher than in the *trnL* intron, and resulted in intraspecific variation as clusters of closely related sequences. Sequence divergences and differences in mitochondrial *COI* and chloroplast *trnL* were more similar at deeper taxa levels, possibly because mtDNA is affected by saturation of nucleotide variation. This may also compromise its power at basal levels of the tree, e.g., however, the precise resolution of pest-host interaction's basal relationships was not of great concern for the current study co-evolutionary analyses were mostly affected by host switches nearer the tips of the trees. Therefore, more critical and robust markers (used in existing phylogenetic datasets) will help to understand the power of the current analyses and improve with denser taxonomic sampling.

The future implementation of this method will benefit from the growing taxonomic coverage in databases and regional genetic botanical inventories, improved match analysis methods that overcome the limitations of BLAST, and the use of multiple marker systems to refine the identification of hosts. These developments will further increase the value that the host plant DNA can be amplified with excellent

reliability from a DNA sample extracted from phytophagous beetles. The technique extends the use of DNA barcoding methods to species identification and co-evolutionary relationships of trophic interactions. Therefore, a metagenome sequencing study is needed to elucidate the multidimensional interaction of pest and host plants so that strategic planning can control the pest insects' damage.

4. 5 Conclusion

The trnl and COI from the coleopteran- host plants using standard extraction protocols provide the information on its evolutionary, ecological, and herbivore–host plant interactions. The current study evaluates the utility of this method when applied to a highly complex group. In contrast, a future increase of the database and use of additional chloroplast and mitochondrial markers will improve the precision, some apparent precincts of host plant inferences independent of the study method. The current inventory concludes that species-specific homology modelling concerning plant host species using trnL and COI accurately deciphers the phylogeny and unravels intraspecies and interspecies divergence nucleotide distance. The documentation of A, C, G, T suggests an increase in AT bias in interspecies association and may play a major in driving the pest towards the host plant. Moreover, it also demonstrates the mutation rates i.e., the occurrence of transition, transversion, synonymous to non-synonymous changes occurring in the mitochondrial, and chloroplast markers. However, detailed analysis using the CYP P450 marker will help us understand the mutation rates generated due to pesticide exposure, which will help us control the pest population.

Feeding studies of agroecological importance to date have not questioned taxonomy and species limits of host plants, nor assessed population differentiation and geographic turnover. DNA-based analyses will contribute vital information on host populations and spatial differentiation of host use due to this more excellent resolution. Therefore, the technique permits the reinvestigation of pertinent hypotheses explaining agro diversity, the factors that influence the community characteristics like density-dependent factors that maintain high diversity, or maybe the correlation of Coleoptera diversity with the phylogeny of host plants. However, the metagenome analysis on the host-plant use and host specificity opens a new avenue for validation above mentioned hypotheses.