Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(6): 165-171

Received: 01-09-2018
Accepted: 03-10-2018

Manjunatha R
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

Shashidhar Viraktamath
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

Ganesha Halikatti
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

Correspondence
Manjunatha R
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

Detection of genetic variation in mango leafhoppers based on mitochondrial marker cytochrome oxidase I (COI) and their phylogenetic relationships of south Indian populations

Manjunatha R, Shashidhar Viraktamath and Ganesha Halikatti

Abstract
Leafhopper is one of the most serious pest of mango which may cause more than 50-60 per cent losses in cases of severe infestation. Molecular characterization of eleven different populations belonging to various mango growing regions of the country was done by using Cytochrome C oxidase I (COI) gene. Molecular analysis of mitochondrial DNA, the leafhopper populations of COI gene sequences were confirmed as *I. nitidulus*, *I. nagpurensis* and *A. atkinsoni*. COI gene sequences of *I. nitidulus* of Raichur, Bramhavar and Hyderabad population showed 99 per cent similarity, *A. atkinsoni* of Dharwad and Shivamogga showed 99 per cent similarity and *I. nagpurensis* of Kerala showed 98 per cent similarity. Dendrogram of indicated all the populations belong to two major clades. Therefore, it is inferred that there was a considerable molecular diversity among the leafhopper populations of major mango growing areas. The maximum identity of *I. nitidulus* and *I. nagpurensis* showed 91 to 99 per cent indicating a higher genetic diversity in these two species and in *A. atkinsoni* the variation showed 97 to 99 per cent.

Keywords: Mango, COI, leafhoppers, genetic diversity

Introduction
Mango (*Mangifera indica* L.) is the most ancient among the tropical fruits and is believed to have originated in the Indo-Burma region. It is in cultivation for more than 4000 years. It is the most popular and choicest fruits of India and occupies a prominent place among the best fruits of the world. It is a good source of sugar, vitamin A and C, calcium and phosphorus. Besides Oriental region, mango is cultivated all over the world. Some of the regions in Australia, Africa and New World actively contribute to the production of mango [15]. Mango, due to its wide adaptability, delicious taste, excellent flavor, high nutritive value and attractive appearance marks itself as king of fruits in India. It is the third largest tropical fruit after banana and citrus in terms of acreage and production in the world. However, in India, it is the major fruit crop occupying an area of about 2.52 million hectares with a production of 18.43 million metric tonnes and productivity of 7.30 MT/ha [1]. It is greatly relished for its succulence, exotic flavour and delicious taste. It enjoys the same popularity in the tropics as an apple in the temperate region. India is the largest producer of choice table varieties of mango in the world. More than 1000 Mango varieties are under cultivation in India, each differing in shape, size and taste [19].

Over 492 species of insects have been reported to infest mango crop [22]. Among the pests that occur on mango, leafhoppers are economically important [6]. A total of 37 species of Auchenorrhyncha in seven families are associated with mango all over the world. These groups form major pest taxa of mango in India. Six subfamilies of Cicadellidae having 26 species are reported to feed on mango leaves and inflorescence. Subfamily Idiocerinae is a predominant group of leafhoppers on mango [23]. The nymphs and adults cluster on the lower side of tender leaves and on inflorescence and suck the sap, resulting in drying of the entire inflorescence and even small fruits and ultimately resulting in yield loss. Nymphs cause more damage than the adults. Besides the direct damage, leafhoppers excrete honeydew, which supports the growth of black sooty mold (*Capnodium mangiferae* Ek.), thus adversely affecting the photosynthetic activity of the plant.

The loss caused by mango leafhoppers, *Amritodus atkinsoni* (Lethierry), *Idioscopus nitidulus* (Lethierry) and I. *clypealis* (Lethierry) was estimated to range from 20-100% of inflorescence [9, 17]. In India, area under mango is rapidly increasing, thus developing into perennial stretches of monocropping system. Consequently this has led to the increased leafhopper incidence. Among various species of mango leafhoppers, *I. clypealis*, *I. nitidulus* and *A. atkinsoni* have been reported as the predominant pests of mango [22]. Later, *Amritodus brevistylus* Viraktamath and *Idioscopus nagpurensis* (Pruthi) were also recorded as pests on mango [16].

The taxonomic tools for identification of insect pest are often laborious, time consuming and requires considerable skill; whereas bio- chemical markers, such as esterase isozymes are influenced by the environment, food plants (hosts) and stage of the insect. In contrast, molecular markers are relatively recent, reasonably accurate and can be complimented with the other methods. Molecular markers are more powerful tools to assess relationships at the population level [5]. Genetic differentiation and gene flow between the species can be analyzed by using various molecular markers likely RAPD, microsatellite, mitochondrial and ribosomal markers [12]. The ΔNA sequencea are used as genetic "barcodes" that may potentially to be used as a bio-identification system for the animals and has proven to be useful identification tool for the vertebrates such as birds [10, 25] and hexapod orders such Lepidoptera [8], Coleoptera [7], Diptera [21], Hymenoptera [20], Ephemereoptera [3] and Hemiptera [13]. At present, there is scanty information available on molecular variation among the south Indian populations of mango leafhopper. Hence, the present investigation was carried out using cytohrome oxidase I (COI) region of mitochondrial DNA to understand the genetic difference in leafhopper populations of major mango growing states of India.

2. Material and Methods

The studies were carried out at the Biotechnology laboratory of the Department of Plant Pathology, UAS, Dharwad during 2014-15. The representatives of leafhoppers populations were collected from different mango growing areas of the country. Genetic diversity was studied using mitochondrial gene coding for sub unit I of cytochrome oxidase (COI) region.

2.1 Collection and storage of insects

Leafhoppers nymphs as well as adults were collected from the mango tree at peak vegetative stage (approximately 50 from each location) and preserved in 5ml capacity plastic vials containing 70 per cent ethyl alcohol from all locations *viz.*, Karnataka (Dharwad, Belgaum, Gadag, Raichur, Shivamogga, Bramhavar and Chintamani), Andhra Pradesh (Hyderabad and Ananthapur), Maharashtra, Kerala and Bihar of the country with samples representing three fields in a village. Total five states were covered during the course of study (Table 2).

2.2 Isolation of genomic DNA from leafhoppers

Genomic DNA was isolated using the CTAB (cetyl-trimethyl-ammonium-bromide) protocol described by [14]. Leafhopper adults and nymphs were ground in pre-warmed 500 µl of CTAB based extraction buffer using sterile micropestle in a 1.5 ml centrifuge tube. The suspension was vertexed and incubated for 45 min at 65°C with intermittent mixing. The sample was cooled to room temperature and centrifuged for 20 min at 13,000 rpm at 4°C. The supernatant was transferred into 1.5 ml centrifuge tube and equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added and repeatedly inverted. The suspension was centrifuged for 15 min at 12,000 rpm at 4 °C and the supernatant was transferred into 1.5 ml centrifuge tube. The DNA was precipitated by adding an equal volume of pre-chilled isopropanol (-20 ºC) and mixing it gently. The suspension was allowed to stand for overnight at -20 ºC and then DNA was pelleted by centrifugation for 20 min at 12,000 rpm at 4 °C. The supernatant was discarded and pellet was washed with 70 per cent ethanol. Later the suspension was centrifuged for 5 min at 4,000 rpm then alcohol was discarded. Finally, the pellet was dried in dry hot bath and re-suspended in 100 µl of $T_{10}E_1$ (Tris-EDTA) solution. 2 µl of RNase (1 mg/ml) was added and incubated at 37 °C for 1 hour and then DNA was stored at -20 °C for further studies.

2.3 DNA (COI region) amplification through Polymerase Chain Reaction (PCR): A fragment of the mitochondrial cytochrome oxidase I (COI) gene was amplified with the primers LCO-1490 (5´-GGTCAACAAATCATAAAGATATTGG-3´) and HCO-2198 (5´-TAAACTTCAGGGTGACCAAAAAATCA-3´) described as conserved primers for invertebrate DNA [5]. The PCR reaction volume (20 µl) contained 13.7 µl water, 2.0 µl dNTP's (2 mM), 2.0 µl Taq buffer (10X), primers (10 pmoles per µl) of 0.5 µl each, 1.0 µl DNA template and Taq polymerase (5 U/µl) of 0.3 µl. PCR amplification conditions for COI gene in mango leafhopper were as follows:

Table 1: PCR amplification conditions for COI gene

Stage	Step	Temperature (ºC)	Duration	No. of cycles
I	Initial Denaturation	95	5 minutes	1
II	Denaturation	94	1 minute	32
	Annealing	57	1 minute	
	Extension	72	2 minutes	
III	Final Extension	72	20 minutes	1
	Hold	4	-	-

2.4 Agarose Gel Electrophoresis: Gel casting plate and comb was washed with water and wiped with 70 per cent alcohol. The two open ends of the gel casting plate were sealed with cellotape and placed on perfectly horizontal levelled platform with comb inside the gel casting plate. Agarose gel (0.8%) was prepared by adding agarose powder to 1X TAE buffer (prepared from 50X TAE buffer). It was boiled until the agarose dissolved completely and then cooled to lukewarm temperature. Ethidium bromide was added to the agarose prior to pouring in the gel plate. After solidification of the agarose, the comb and cellotape were removed carefully and the casted gel was placed in the electrophoresis unit with wells facing towards the cathode and submerged with 1X TAE buffer to a depth of about 1 cm.

2.5 Loading the DNA samples for confirmation: A piece of parafilm was placed on the solid surface and 7 µl of genomic DNA was pipetted out on to a parafilm. To the DNA samples, 3 µl loading dye (bromophenol blue) was added and mixed thoroughly by pipetting up and down several times gently. The contents were loaded into wells carefully with the help of micropipette. DNA ladder (100 bp) was loaded as standard marker. Cathode and anode were connected to a power pack and gel was run at the constant voltage (70 V). The gel was

run for 60 min until the tracking dye reached half of the gel and bands were visualized and documented in the gel documentation system (UV Doc Image Master® VDS).

2.6 Separation of amplified products by Agarose gel electrophoresis: About 5 µl of amplified products from each tube along with 2 µl loading dye were separated on 1 per cent agarose gel along with 100 bp DNA ladder and gel image was visualized and documented in the gel documentation system.

2.7 Gel elution of PCR fragment: The intense band of 700 base pair PCR products obtained using specific primers were excised from agarose gel with a sterile sharp scalpel blade by keeping the gel on low intensity UV transilluminator. The agarose gel piece containing the product was collected in a sterile micro centrifuge tube.

2.8 Gel purification of PCR fragment and quantification: The excised PCR fragments were subjected for QIAGEN MinElute® PCR purification kit to elute the PCR product from agarose gel. The purification of PCR product was done as described in the user's manual. The purified PCR product was quantified by using the nanodrop.

2.9 Sequencing of PCR product: The fragment of the cytochrome oxidase I (COI) gene of about 700 base pair amplicon was sequenced by using both forward (LCO-1490) and reverse (HCO-2198) primers by commercial sequencing centre, Bangalore Merck (India) Pvt. Ltd., Bangalore using the ABI 3130 XL sequencer.

2.10 Sequence analysis: The sequences obtained using forward and reverse primers were assembled using Vector NTI software. The sequences were subjected to BLAST at http://www.ncbi.nlm.nih.gov. Also DNA sequences were aligned using MEGA 5.0.2.

2.11 Sequence similarity analysis: Dendrogram was constructed using unweighted pair-group arithmetic average (UPGMA) method available in MEGA 4.0.2 software for the nucleotide sequences of COI region of all eleven samples. Published sequence of mango leafhoppers COI gene (HQ268816.1, HQ268815.1 and HQ268819.1) was downloaded from NCBI (National Center for Biotechnology Information) and used in the sequence similarity analysis.

3. Result and Discussion

The sequences obtained using forward and reverse primers were assembled using the Vector NTI software. Nucleotide sequences of 20 test population which comprised three species of leafhoppers *viz., I. nitidulus, I. nagpurensis* and *A. atkinsoni* COI region were analyzed using bioinformatics tools like NCBI (National Center for Biotechnology Information) BLAST programme. Blast analysis of COI gene sequences of 20 test insects revealed that, out of 20 samples, 11 were *I. nitidulus*, five samples were *I. nagpurensis* and four samples were *A. atkinsoni* of cytochrome oxidase subunit I (COI) gene of partial cytochrome oxidase of mitochondria. The blast results of 20 samples with reference gene bank accessions have been represented in Table 2.

3.1 *Idioscopus nitidulus*: Fragment of COI gene sequences of *I. nitidulus* of Raichur, Bramhavar and Kerala showed 99 per cent similarity with NCBI published sequence of *I. nitidulus* COI gene (HQ268816.1). Leafhoppers from Shivamogga showed 98 per cent similarity, while Gadag, Bihar and Kerala showed 95 per cent similarity (Table 2). Samples from Chintamani and Ananthapur showed 91 per cent similarity, while Belagavi showed 93 per cent similarity.

3.1.1 *Idioscopus nagpurensis:* Fragment of COI gene sequences of *I. nagpurensis* from Dharwad and Belagavi population showed 95 per cent similarity with NCBI published sequence of *I. nagpurensis* COI gene (HQ268815.1). Leafhoppers from Kerala, Shivamogga, Belagavi showed 98, 97 and 95 per cent similarity, respectively (Table 2).

3.1.2 *Amritodus atkinsoni:* Fragment of COI gene sequences of *A. atkinsoni* of Dharwad and Shivamogga population showed 99 per cent similarity with NCBI published sequence of *A. atkinsoni* COI gene (HQ268819.1), whereas, that from Belagavi and Maharashtra population showed 98 and 97 per cent similarity, respectively (Table 2).

3.2 Sequence similarity analysis

Dendrogram was constructed using unweighted pair-group arithmetic average (UPGMA) method for the nucleotide sequences of COI region of all 20 mango leafhopper population. Published sequence of *I. nitidulus, I. nagpurensis* and *A. atkinsoni* COI gene (HQ268816.1, HQ268815.1 and HQ268819.1) from NCBI was used in the construction of dendrogram (Figure 3). Phylogenetic tree of 20 different populations of mango leafhopper sequence for COI region formed into two major clusters A and B. Among the two clusters, cluster A was the major comprising 15 populations. Further cluster A formed two sub clusters A1 and A2. A1 comprised 11 populations of *I. nitidulus* from Bramhavar, Kerala, Raichur andhra Pradesh (Ananthapur), Belagavi, Gadag, Dharwad andhra Pradesh (Hyderabad), Shivamogga, Bihar and Chintamani. Sub cluster A2 comprised four populations of *A. atkinsoni* from Maharashtra, Dharwad, Shivamogga and Belagavi. Whereas, cluster B comprised five populations of *I. nagpurensis* from Belagavi, Bramhavar, Kerala, Shivamogga and Dharwad along with the reference sequence of *I. nitidulus, I. nagpurensis* and *A. atkinsoni* COI gene (HQ268816.1, HQ268815.1 and HQ268819.1, respectively). Among cluster A1 populations of *I. nitidulus* from Bramhavar, Kerala and Raichur were closely related with Andhra Pradesh (Ananthapur) and Belagavi populations which were closely inter related with Gadag and Dharwad populations. *I. nitidulus* from Andhra Pradesh (Hyderabad), Shivamogga, Bihar and Chintamani populations were interrelated with cluster A2 populations of *A. atkinsoni* from Maharashtra, Dharwad, Shivamogga and Belagavi forming a separate sub cluster. In cluster B, *I. nagpurensis* from Belagavi and Bramhavar were interrelated with Kerala, Shivamogga and Dharwad population which were closely related with the published sequence of *A. brevistylus.*

3.3 Phylogenetic analysis

3.3.1 *Idioscopus nitidulus:* Phylogenetic tree of 11 populations of *I. nitidulus* sequence for COI region formed into a single cluster. In cluster, populations of Bramhavara, Kerala and Raichur were closely related with Andhra Pradesh (Ananthapur) and Belagavi which are inter related with Andhra Pradesh (Hyderabad) and Shivamogga populations (Figure 2).

3.3.2 ***Amritodus atkinsoni*:** Phylogenetic tree of four populations of *A. atkinsoni* sequence for COI region formed into a single cluster. In the cluster, populations of Shivamogga and Belagavi were closely interrelated with Dharwad and Maharashtra leafhopper populations (Figure 4).

3.3.3 ***Idioscopus nagpurensis:*** Phylogenetic tree of five population of *I. nagpurensis* sequence for COI region formed into two clusters A and B. Cluster A consisted of Shivamogga and Dharwad populations which were closely related with *I. nagpurensis* from Kerala. Whereas, Cluster B, consisted of *I. nagpurensis* from Belagavi and Bramhavar leafhopper populations (Figure 5).

3.3.4 ***F-coefficient analysis:*** The pair wise population F_ST values ranged from a minimum of 0.008 between *A. atkinsoni* from Shivamogga and Dharwad to a maximum of 1.238 between *A. atkinsoni* of Shivamogga and *I. nagpurensis* of Shivamogga. The pair wise F_ST values indicated the presence of moderate to high genetic differentiation among the sampled populations (Table 3).

A DNA based identification system involving the analysis of sequence diversity in the 5´ region of the mitochondrial gene; cytochrome oxidase 1 has been widely used for studying the genetic differentiation and evolution in the animal systems [11]. The cytochrome oxidase I (COI) region of mtDNA is the most studied region of the insect mitochondrial genome. The COI region is regularly used as a global identification system (DNA Barcoding) for insects and other animals [18]. COI region is commonly employed as molecular markers and they have already proved to be useful for separating distant groups of individuals within an insect species [4]. In the present study, attempt was made to study the genetic diversity of mango leafhoppers by amplifying approximately 700 base pair of cytochrome oxidase sub unit I (COI) from mango leafhopper adults of major mango growing areas. The information obtained from these studies gave more useful tips for assessing the genetic diversity. Nucleotide sequences of 20 mango leafhoppers test populations which comprised three species of leafhoppers *viz., I. nitidulus, I. nagpurensis* and *A. atkinsoni* COI region were analyzed using bioinformatics tools like NCBI (National Center for Biotechnology Information) BLAST programme. Blast analysis of COI gene sequences of 20 test insects revealed that, 11 samples were *I. nitidulus,* five samples *I. nagpurensis* and four samples were *A. atkinsoni*. Similarly, [2] barcoded five species of mango leafhoppers at IIHR *viz., A. atkinsoni*, *A. brevistylus*, *I. clypealis*, *I. nagpurensis* and *I. nitidulus*. Phylogenetic tree of 20 different populations of mango leafhopper sequence for COI region formed two major clusters A and B. Among the two clusters, cluster A was the major comprising 15 populations. Further, cluster A formed two sub clusters A1 and A2. A1 comprised 11 populations of *I. nitidulus* from Bramhavar, Kerala, Raichur andhra Pradesh (Ananthapur), Belagavi, Gadag, Dharwad Andhra Pradesh (Hyderabad), Shivamogga, Bihar and Chintamani. Sub cluster A2 comprised four populations of *A. atkinsoni* from Maharashtra, Dharwad, Shivamogga and Belagavi. Whereas, cluster B comprised five populations of *I. clypealis* from Belagavi, Bramhavar, Kerala, Shivamogga and Dharwad along with the reference sequence of *I. nitidulus, I. nagpurensis* and *A. atkinsoni* COI gene (HQ268816.1, HQ268815.1 and HQ268819.1, respectively). A glance towards dendrogram revealed that, there is diversity among three different mango leafhopper populations of both inter and intra specific populations of twenty locations. The pair wise F_ST values indicated the presence of moderate to high genetic differentiation among the sampled populations. The genetic differences are probably induced by the location pressure due to variations in the insecticide usage, diversity in the use of mango cultivars and cultural practices. The maximum identity of *I. nitidulus* and *I. nagpurensis* varied from 91 to 99 per cent with reference to the sequences deposited in gene bank of NCBI indicating a higher genetic diversity in these two species. However, in case of *A. atkinsoni* the variation was only 97 to 99 per cent.

The present study concluded that the genetic differences are probably induced by the location pressure due to variations in the insecticide usage, diversity in use of mango cultivars and cultural practices. In conclusion, molecular evidence offered here new insight in understanding mango leafhopper populations. There is need for exploring other markers like simple sequence repeats (SSR) or microsatellites for future studies and also further research in understanding biotype development.

Table 2: Comparison and identity of mango leafhoppers from different places

Sl No.	Mango leafhopper collected during 2014-15 (collected place)	Identified as	Per cent Homology	Accession number (Referred gene bank)	Species	Reference
			Idioscopus nitidulus			
1	Dharwad	*Idioscopus nitidulus*	91	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
2	Belagavi	*Idioscopus nitidulus*	93	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
3	Raichur	*Idioscopus nitidulus*	99	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
4	Shivamogga	*Idioscopus nitidulus*	98	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
5	Chintamani	*Idioscopus nitidulus*	91	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
6	Gadag	*Idioscopus nitidulus*	95	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
7	Bramhavar	*Idioscopus nitidulus*	99	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
8	Bihar	*Idioscopus nitidulus*	95	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
9	Hyderabad (AP)	*Idioscopus nitidulus*	95	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
10	Kerala	*Idioscopus nitidulus*	99	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
11	Ananthapur (AP)	*Idioscopus nitidulus*	91	HQ268816.1	IIHR Bt-06	Veda Prasanna *et al.*, 2010 [24].
			Idioscopus nagpurensis			
12	Shivamogga	*Idioscopus nagpurensis*	97	HQ268815.1	IIHR Bt-05	Veda Prasanna *et al.*, 2010 [24].
13	Dharwad	*Idioscopus nagpurensis*	95	HQ268815.1	IIHR Bt-05	Veda Prasanna *et al.*, 2010 [24].
14	Kerala	*Idioscopus nagpurensis*	98	HQ268815.1	IIHR Bt-05	Veda Prasanna *et al.*, 2010 [24].
15	Belagavi	*Idioscopus nagpurensis*	95	HQ268815.1	IIHR Bt-05	Veda Prasanna *et al.*, 2010 [24].
16	Bramhavar	*Idioscopus nagpurensis*	91	HQ268815.1	IIHR Bt-05	Veda Prasanna *et al.*, 2010 [24].
			Amritodus atkinsoni			
17	Dharwad	*Amritodus atkinsoni*	99	HQ268819.1	IIHR Bt-09	Veda Prasanna *et al.*, 2010 [24].
18	Shivamogga	*Amritodus atkinsoni*	99	HQ268819.1	IIHR Bt-09	Veda Prasanna *et al.*, 2010 [24].
19	Maharashtra	*Amritodus atkinsoni*	97	HQ268819.1	IIHR Bt-09	Veda Prasanna *et al.*, 2010 [24].
20	Belagavi	*Amritodus atkinsoni*	98	HQ268819.1	IIHR Bt-09	Veda Prasanna *et al.*, 2010 [24].

Table 3: Pair wise estimates of evolutionary divergence between sequences of mango leafhoppers

	InBGM	InRCR	InGDG	InBRM	InKRL	InAPA	InSHG	InCHM	InDWD	InAPH	InBHR	INKRL	INSHG	INDWD	INBGM	INBRM	AaDWD	AaSHG	AaMH	AaBGM
InBGM	0																			
InRCR	0.027	0																		
InGDG	0.077	0.047	0																	
InBRM	0.027	0.000	0.047	0																
InKRL	0.027	0.000	0.047	0.000	0															
InAPA	0.044	0.016	0.064	0.016	0.016	0														
InSHG	0.069	0.056	0.108	0.056	0.056	0.040	0													
InCHM	0.184	0.149	0.208	0.149	0.149	0.150	0.200	0												
InDWD	0.077	0.047	0.00	0.047	0.047	0.064	0.108	0.208	0											
InAPH	0.078	0.048	0.099	0.048	0.048	0.040	0.073	0.194	0.099	0										
InBHR	0.095	0.073	0.126	0.073	0.073	0.091	0.118	0.236	0.126	0.118	0									
INKRL	1.006	0.996	1.06	0.996	0.996	0.996	1.034	0.972	1.060	0.996	1.054	0								
INSHG	1.061	1.050	1.106	1.050	1.050	1.050	1.091	1.025	1.106	1.084	1.113	0.036	0							
INDWD	1.030	1.020	1.092	1.020	1.020	1.020	1.059	1.038	1.092	1.053	1.080	0.056	0.019	0						
INBGM	0.987	0.978	1.028	0.978	0.978	0.978	1.014	0.970	1.028	1.008	1.034	0.065	0.044	0.065	0					
INBRM	1.021	1.011	1.046	1.011	1.011	1.011	1.049	1.031	1.046	1.049	1.07,0	0.086	0.065	0.086	0.019	0				
AaDWD	0.235	0.213	0.272	0.213	0.213	0.224	0.269	0.348	0.272	0.241	0.275	1.152	1.218	1.180	1.104	1.114	0			
AaSHG	0.24	0.224	0.284	0.224	0.224	0.235	0.281	0.361	0.284	0.252	0.286	1.170	1.238	1.199	1.121	1.131	0.008	0		
AaMH	0.251	0.235	0.284	0.235	0.235	0.246	0.292	0.373	0.284	0.263	0.298	1.152	1.218	1.180	1.104	1.114	0.023	0.016	0	
AaBGM	0.240	0.224	0.284	0.224	0.235	0.281	0.361	0.284	0.252	0.286	1.17,1	0.238	1.199	1.121	1.131	0.008	0.000	0.016	0.016	0

In – *Idioscopus nitidulus* Aa- *Amritodus atkinsoni* IN- *Idioscopus nagpurens*

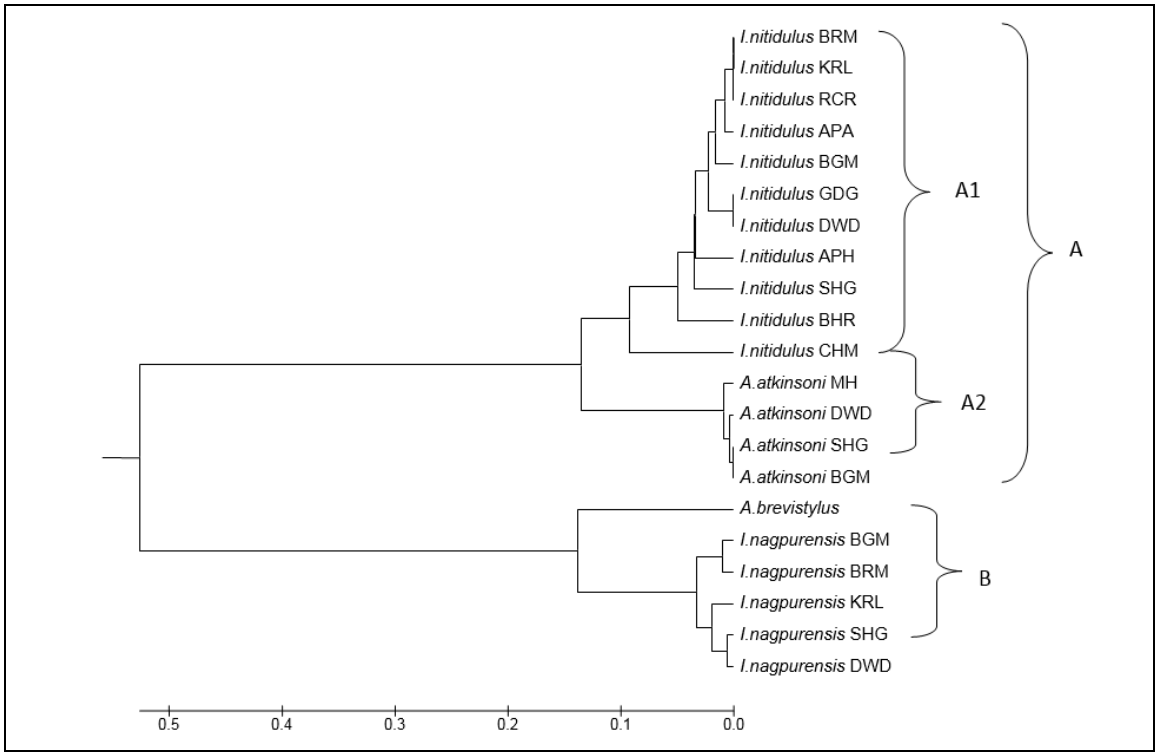

Fig 1: Dendrogram showing relationships among leafhopper species from different places

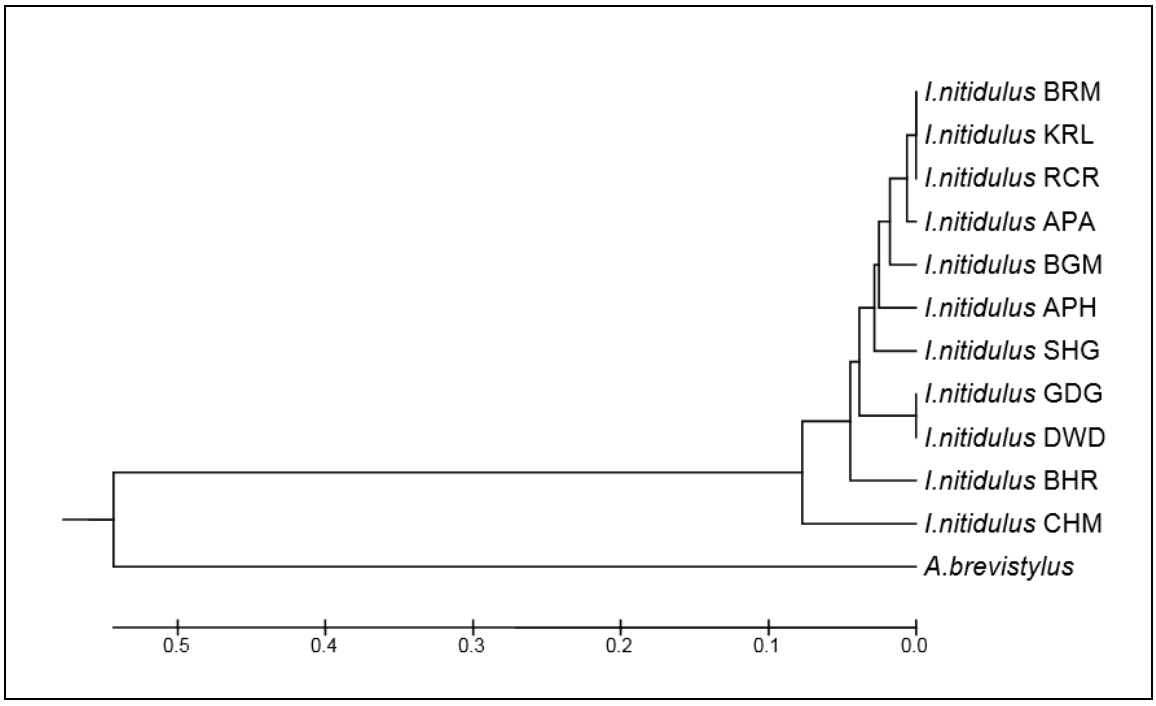

Fig 2: Dendrogram showing relationships among *Idioscopus nitidulus* from different places

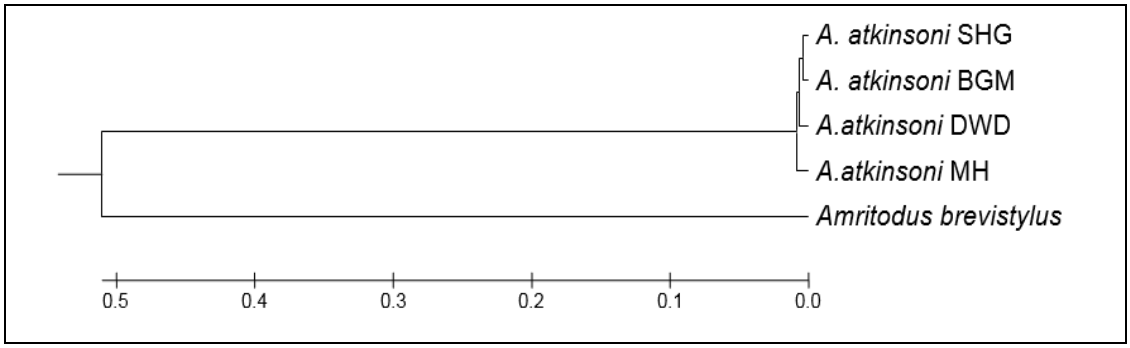

Fig 3: Dendrogram showing relationships among *Amritodus atkinsoni* from different places

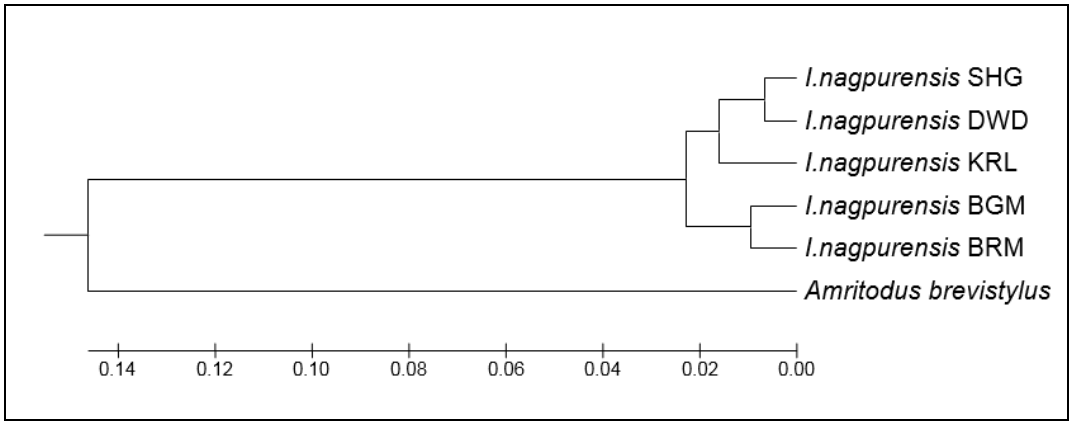

Fig 4: Dendrogram showing relationships among *Idioscopus nagpurensis* from different places

4. Acknowledgment
Senior author thankfully acknowledges Institute of Agriculture Biotechnology (IABT) and Department of Plant Pathology, University of Agricultural Sciences, Dharwad for facilitating the work.

5. References

1. Anonymous. Area and Production of Horticultural Crops, Bulletin of National Horticulture Board, India, 2014.
2. Asokan R, Sunil Joshi, Krishna Kumar NK, Rebijith KB. DNA barcode for important sap sucking pests of horticultural crops. Indian Institute of Horticulture Research Technical Bulletin. 2011; 37(3):40-44.
3. Ball SL, Hebert PDN, Burian SK, Webb JM. Biological identification of mayflies (Ephemeroptera) using DNA barcodes. Journal of the North American Benthological Society. 2005; 24:508-511.
4. Behura SK. Molecular marker systems in insects: Current trends and future avenues. Molecular Ecology Resources. 2006; 15:3087-3113.
5. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 1994; 3:294-299.
6. Gangolly SR, Singh R, Katyal SL, Singh D. *Mango*, ICAR, New Delhi, 1957, 492.
7. Greenstone MH, Rowley DL, Heimbach U, Lundgren JG, Pfannenstiel RA, Rehner SA. Barcoding generalist predators by polymerase chain reaction: carabids and spiders. Molecular Ecology. 2005; 14:3247-3257.
8. Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Herbet PDN. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of National Academy of Sciences. 2006; 103:968-972.
9. Haseeb M. Occurrence of fruit sucking bug on mango. Annuls of Plant Protection Sciences. 2006; 14:218-219.
10. Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. Identification of birds through DNA barcodes. PLoS Biology. 2004; 2:1657.
11. Hebert PD, Rowan DN. Identifying the spiders through DNA barcodes. Canadian Journal of Zoology. 2005; 83(3):481-491.
12. Kim I, Bae JS, Choi KH, Jin BR, Lee KR, Sohn HD. Haplotype diversity and gene flow of diamond back moth, *Plutella xylostella* (L.) (Yponomeutidae: Lepidoptera) in Korea. Korean Journal of Applied Entomology. 2000; 39:43-52.
13. Lee W, Kim H, Lim J, Choi H, Kim Y, Yanh-Su K *et al*. Barcoding aphids (Hemiptera: Aphididae) of the Korean Peninsula: Updating the global data sheet. Molecular Ecology Resources. 2011; 11:32-38.
14. Marzachi C, Veratti F, Bosco D. Direct PCR detection of phytoplasmas in experimentally infected insects. Annals of Applied Biology. 1998; 133:45-49.
15. Mukheerjee SK. Origin of Mango. (*Mangifera indica*). Ecology Botany. 1972; 26:260-266.
16. Pingale RD, Patil SP. Preliminary trial on chemical control of mango hoppers (*Amritodus atkinsoni* and *Idioscopus nitidulus*) in laboratory. Indian Journal of Agricultural Sciences. 1988; 58:502-503.
17. Rahman S, Kuldeep MA. Mango hopper: Bio-ecology and management- A review. Agriculture Review. 2007; 28:49-55.
18. Saraste M. Structural features of cytochrome oxidase. Review. Biophysical Journal. 1990; 23:331-336.
19. Singh RN. Mango. Indian Council of Agricultural Research: New Delhi, India, 1990.
20. Smith MA, Rodriguez JJ, Whitefield JB, Andrew RD, Janzen DH, Winnie H *et al*. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections, PNAS, 2008; 105:12359-12387.
21. Smith MA, Wood DM, Janzen DH, Hallwachs W, Hebert PDN. DNA barcodes affirm that 16 species of apparently generalist trophical parasitoid flies (Diptera: Tachanidae) are not all generalists. Proceedings of National Academy of Sciences. 2007; 104:4967-1972.
22. Tandon PL, Verghese A. World list of insects, mite and other pests of mango. *Tech. Bull.*, Indian Institute of Horticulture Research. 1985; 5:22-26.
23. Viraktamath CA. Auchenorrhyncha (Homoptera) associated with mango, *Mangifera indica* L. Tropical Pest Management. 1989; 35:431-435.
24. Veda Prasanna, Devi PN, Chaitanya BN, Asokan R, Rebijith KB, Ellango R *et al*. Molecular diversity of mango leafhoppers, *NCBI* deposit, IIHR, Bangalore, 2010.
25. Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. DNA barcoding Australian fish species. Philosophical Transactions of the Royal Society B Biological Sciences. 2005; 360:1847.