DNA BARCODING OF SPIDER MITES (PROSTIGMATA: TETRANYCHIDAE) ASSOCIATED WITH ORNAMENTAL PLANTS

by
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(2017-11-005)

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COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
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THESIS

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CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY
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KERALA, INDIA

2019

DECLARATION

I hereby declare that this thesis entitled **"DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants"** is a record of research work done independently by **Ms. Jayalakshmi Prakash** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the advisory committee of **Ms. Jayalakshmi Prakash** (2017-11-005), a candidate for the degree of Master of Science in Agriculture, with major field in Plant Biotechnology, agree that this thesis entitled **"DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants"** may be submitted by **Ms. Jayalakshmi Prakash**, in partial fulfillment of the requirement for the degree.

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JAYALAKSHMI PRAKASH

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ABBREVIATIONS

%	Percentage
µg	Microgram
µl	Microlitre
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide basic local alignment search tool
bp	Base pair
CPBMB	Centre for Plant Biotechnology and Molecular Biology
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribo Nucleoside Triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
g	Gram
L	Litre
M	Molar
MEGA	Molecular evolutionary genetics analysis
mg	Milligram
ml	Millilitre
mM	Millimolar
NCBI	National Centre for Biotechnology Information
ng	Nanogram
^{o}C	Degree Celsius
OD	Optical Density
PCR	Polymerase Chain Reaction
pH	Hydrogen ion Concentration
ITS2	Second Internal Transcribed Spacer

COI	Mitochondrial Cytochrome Oxidase subunit I
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
rpm	Revolutions per minute
TAE	Tris Acetate EDTA
TE	Tris EDTA
UV	Ultra violet
V	Volts
BOLD	Barcode of Life Database System


Introduction

1. INTRODUCTION

Horticultural crop production adds a significant share to the total agricultural output of the nation. Total area under floriculture in India is estimated to be 0.31 million hectares with a production of 2.08 million tonnes loose flowers and 0.82 million tonnes cut flowers in 2018-19 (IndiaAgristat, 2019). In Kerala, Thrissur district is considered as the centre of floriculture nursery business. Majority of nurseries in the area do not maintain their own sources of mother plants. They purchase plants from other states or import planting materials from other countries. Trade of commercial ornamentals has been recognized worldwide as an important invasion pathway for non-native pests. Recently, two species of phytophagous mites were reported for the first time in India from commercial nurseries in Thrissur district, Kerala. These mite pests *viz*., *Tenuipalpus pacificus* Baker on orchid (Bhaskar *et al*., 2013) and *Tetranychus okinawanus* Ehara on *Adenium obesum* (Forssk.) Roem. & Schult (Zeity *et al.,* 2016) would probably have gained entry into India through imported planting materials. The mite, *T. okinawanus* has now emerged as a predominant species of mite pest on different crops of Kerala. Recent record of *T. okinawanus* on cucumber, banana and five other host plants from Thrissur and neighbouring districts indicates that this alien species has potential to turn invasive (Arunima *et al*., 2017).

The family, Tetranychidae comprising of spider mites, is one of the most important of the Acari in terms of economic impact, because it comprises several agricultural pest species of major relevance (Migeon *et al*., 2010). Spider mites are the most important among the mite pests which include 1,321 valid species, 16,221 host records on 3,917 different plants and 8,063 geographic distribution records (Spider Mites Web, 2019). Spider mites are highly polyphagous in nature and due to its short life cycle, high fecundity and small size, its control is cumbersome. They are the most diverse group of arthropods encountered in quarantines. Many spider mite species intercepted at the port of entry belonged to the genus *Tetranychus viz*., *T. evansi* Baker & Pritchard *T. fijiensis* Hirst and *T.*

kanzawai Kishida, because of the intercontinental movement of fruits, flowers and ornamental plants (Dhooria, 2016).

Though several species of mites were reported on various commercial ornamental plants from different parts of India, no systematic study has been conducted so far to document the fauna of spider mites associated with ornamental plants in Kerala. Morphological identification of species of Tetranychidae is difficult, due to morphological similarity between species. Species delineation cannot be based on external morphology alone in taxa where cryptic species exists, and molecular taxonomy can act as reinforcement to the conventional taxonomy. DNA barcoding method using the sequence diversity of mitochondrial *COI* gene has been established as a taxonomic system to study animal species diversity. This technique has been effectively utilised for species identification of spider mites by many workers (Bennur *et al*., 2015; Arabuli and Gotoh, 2018). Development of DNA barcoding technique and availability of species specific markers triggered even non-taxonomists to identify cryptic spider mite species. In this background, the present study was proposed to document the diversity of spider mites infesting commercial ornamental plants of Thrissur and Ernakulam districts of Kerala with the following objectives:

1. To generate DNA barcodes for different species of spider mites infesting commercial ornamental plants of central Kerala
2. To find out the genetic variability among them

Review of Literature

2. REVIEW OF LITERATURE

The members of the family Tetranychidae, commonly called as spider mites are the most diverse among the phytophagous mites. The family consists of most injurious plant feeding mite pests of agricultural and ornamentals worldwide (Bolland *et al.*, 1998; Zhang, 2003; Hoy, 2011).

Under optimum conditions, spider mites can complete their development from egg to adult in less than one week, so there may be many overlapping generations in a single season. Therefore, populations can increase rapidly and cause extensive plant damage in a very short time. Reproductive potential of spider mites is very high with seven to ten generations in a single growing season during hot, dry weather conditions. It completes one generation within four to fourteen days with faster rate of development, above 91°F. Mite outbreaks are severe during dry weather conditions. Mites spin webs using pedipalp and they spread to new host plants and fields by these silken webs caught on breeze (Cullen and Schramm, 2009).

2.1. Spider mite pest of ornamental plants

The common red spider mite, *Tetranychus telarius* (Linn.) and *Tetranychus equatorius* (McGregor) species are extremely polyphagous and occur on a large number of plants which include common vegetable and ornamental plants like brinjal, tomato, rose, jasmine, gourds, *etc*. (Cherian, 1931).

Metcalf and Flint (1962) reported that many ornamentals and vegetable crops along the Pacific coast of U. S. A., were attacked by the Pacific mite, *Tetranychus pacificus* McGregor, species which was often very destructive, resulting in the total failure of crops. Sepasgozarian (1971) reported the infestation of spider mites *Tetranychus urticae* Koch and *Tetranychus cinnabarinus* Boisduval on ornamental plants in Iran. Manesh (1972) reported *T. cinnabarinus* and *Tetranychus cucurbitae* Rahman and Sapra on China rose.

Prione (1978) had discussed about two spotted red spider mites on ornamental plants in his book 'Diseases and Pests of Ornamental Plants'. The mite which was favoured by warm dry weather condition, infest a wide variety of plants growing in greenhouses. The spider mite species, *Tetranychus truncatus* Ehara was reported for the first time in India on ornamental plant, *Dahlia* sp. from Northwestern Himalayan regions of Jammu and Kashmir and Himachal Pradesh by Rather (1983).

Van de Vrie *et al*. (1985) reported that the two-spotted spider mite, *Tetranychus urticae* Koch was the major pest of greenhouse roses (*Rosa* sp.), a highly significant crop in Mexico. Sood and Kakar (1990) reported *Tetranychus ludeni* Zacher on ornamental plant, *Dahlia* from India. Dhooria (1999a) recorded *Euteranychus orientalis* Klein as a major pest of ornamental plants in Punjab. Dhooria (1999b) also reported *T. urticae* as a serious pest on roses grown in polyhouses in Punjab. The spider mite, *T. urticae* infestation was also recorded on rose grown in open condition in Kerala (Onkarappa *et al*., 1999).

The mite, *T. urticae* was reported to feed on ornamental plants in both polyhouse and field conditions due to the changing agricultural scenario (Natarajan, 2001). In Hawaii, carmine spider mite, *T. cinnabarinus* is a serious pest on the ornamentals *viz*., carnation, chrysanthemum, cymbidium, gladiolus, marigold and rose (Mersino, 2002). Zhang (2003) reported the incidence of two spotted spider mite, *T. urticae* on greenhouse ornamentals *viz.*, rose, carnation and gerbera and *T. cinnabarinus* mite on greenhouse carnation in his book 'Mites of greenhouses'.

Eighty one species of mites belonging to 11 families including two spider mites *viz*., *T. urticae* and *Tetranychus* sp. collected on ornamental plants shipped from Guatemala to Miami International airport in Florida. These mites recovered from a variety of ornamental plant genera, are potentially serious pests (Childers and Rodrigues, 2005).

Sharma (2005) studied the seasonal incidence of *T. urticae* on rose plants grown under polyhouse condition, and found two peak periods of infestation, June and October. Pal and Sarkar (2009) recorded red spider mite, *T. urticae* on ornamental plants *viz*., carnation, gerbera and chrysanthemum growing in hilly regions of West Bengal during summer months.

Seasonal abundance of spider mite, *T. urticae* on ornamental plants *viz.*, bougainvillea, celosia, chrysanthemum, dahlia, hibiscus, ixora, jasmine, daisy, mussaenda, rose and zinnia was studied by Haque *et al*. (2011) from Rajshahi. The study revealed that the peak mite population prevailed during the month of August and the mite population had significant positive correlation with temperature. Singh and Raghuraman (2011) reported the spider mite, *Tetranychus neocaledonicus* Andre on ornamental plants and vegetable crops in North India.

Haghghadam and Arbabi (2012) reported mites of family, Tetranychidae on ornamental plants from Guilan and West of Mazandaran, Iran. Six species of spider mites *viz*., *Tetranychus urticae*, *T. ludeni*, *T. roseus*, *Oligonychus bicolar*, *Petrobia phacelia* and *P. latens* infesting ornamental plants *Alocasia* sp., *Dieffenbachia amoena*, *Acalypha hispida*, *Impatiens* spp., *Aspidistra elation, Chamaedorea elegans, Oxalis priangularis* and *Fittonia verschaffeltn* were recorded. In Japan, Matsuda *et al.* (2013) reported the occurrence of *Tetranychus piercei* McGregor on *Ipomea indica* from Okinawa and *T. urticae* on carnation from Nagano and Kanagawa.

Binisha and Bhaskar (2013) studied the diversity of spider mites associated with ornamental plants in Thrissur district in Kerala and recorded spider mite species under three genera *viz.*, *Tetranychus, Oligonychus* and *Eutetranychus*. Jagadish *et al*. (2013) reported the spider mite, *Tetranychus ludeni* Zacher on sunflower for the first time in India. Shah and Shukla (2014) studied the seasonal incidence of two spotted spider mite, *T. urticae* on gerbera grown under polyhouse. Infestation was found to be continued throughout the year. They also found that the mite population was significantly high on top strata of plants.

Singh and Chauhan (2014) reported the spider mite, *T. urticae* on rose, marigold, antirrhinum and carnation, *T. ludeni* Zacher on rose, antirrhinum and carnation and *Tetranychus hypogeae* Gupta and *Tetranychus hydrangeae* Pritchard and Baker on primrose and weigella from different localities in mid-hill region of Himachal Pradesh. The study reported *T. hypogeae* and *T. hydrangeae* for the first time in the state. Singh (2015) recorded four species of phytophagous Tetranychid mites *viz*., *T. hypogeae* on prime rose; *T. ludeni* on rose, carnation, antirrhinum and prime rose; *T. neocaledonicus* on nigella and *T. urticae* on rose, carnation, marigold, dahlia, calendula and jasmine from Himachal Pradesh.

Dhooria (2016) reported that the spider mite species *Tetranychus evansi*, *T. fijiensis*, *T. kanzawai* and *T. pacificus* were intercepted at the port of entry because of the intercontinental movement of fruits, flowers and ornamental plants. The spider mite, *T. okinawanus* native to Okinawa Island of Japan was reported in India for the first time on *Adenium obesum* recently (Zeity *et al.*, 2016). Arunima (2017) reported the spider mite, *Tetranychus truncatus* Ehara on *Dahlia* from Idukki district of Kerala. According to Desai *et al*. (2017), spider mite *Tetranychus urticae* Koch is serious pest on rose grown in polyhouse and open conditions in Navsari, Gujarat.

Karmakar (2018) took up a comprehensive study on mites occurring on 26 types of ornamental and floricultural plants and 22 types of fruit trees covering nine districts of south Bengal and reported the infestation of *T. urticae* on rose, chrysanthemum, zinnia, carnation, tube-rose, marigold, dahlia, sunflower, gladiolus; *T. neocaledonicus* on jasmine, rose, geranium; *T. ludeni* on cosmos, marigold; *Eutetranychus orientalis* on rose, bougainvillea, sunflower and *Oligonychus biharensis* Hirst on *Plumeria alba*.

2.2. DNA barcoding for species determination

Din and Engberg (1979) established that conservative DNA molecules from different species are easily distinguishable through restriction enzyme analysis of cytoplasmic rDNA from macromolecules. Nanney (1982) had

predicted that with the advance of technology, organisms can be distinguished on the basis of difference in bases of DNA sequence. He also found that phenotype and genotype of species could be compared using molecular foundation of those characteristics.

Folmer *et al*. (1994) described universal primers of mitochondrial *cytochrome c oxidase subunit I* gene of eleven invertebrate phyla: Echinodermata, Mollusca, Annelida, Pogonophora, Arthropoda, Nemertinea, Echiura, Sipuncula, Platyhelminthes, Tardigrada, Coelenterata and Vestimentifera and reported that COI primers can produce informative sequences for phylogenetic analysis. Blouin *et al.* (1998) examined the usefulness of mtDNA for nematode phylogeny reconstruction and also provide data that can be used for a priori character weighting or for parameter specification in models of sequence evolution.

DNA sequence data can be used not only to assess genetic distances, but also to seek phylogenetic relationships between populations, to plot the evolutionary history of the species and to suggest hypotheses concerning its centre of origin. When the overall structure of a species is examined, a distinction must be made between its geographical structure and host-related structure (Navajas, 1998)

In molecular systematics mitochondrial genes are widely used, because of their high copy number than single copy nuclear genes and their maternal inheritance. These features are particularly useful at the intraspecific level (Navajas and Fenton, 2000). Anderson and Trueman (2000) conducted study on bee parasitic mite, *Varroa jacobsoni* collected from *Apis cerana* and found that *COI* sequences formed two different clades in phylogenetic analysis. This revealed that *V. jacobsoni* is a complex of two different species.

Trewick (2000) conducted experiment to find species of *Peripatus* collected from 54 different locations throughout New Zealand. Out of the collected 157, he found 62 haplotypes by analysing the mitochondrial *cytochrome oxidase I* (*COI*) diversity among the individuals. Eggert *et al.* (2002) conducted

experiments to find the genetic differences between the forest elephants, *Loxodonta cyclotis* and savannah elephants, *Loxodonta africana* of West and Central Africa by examining mitochondrial *cytochrome b* sequences and microsatellite loci. Floyd *et al.* (2002) developed a molecular operational taxonomic unit (MOTU) scheme for soil nematodes using a molecular barcode derived from sequencing of the 5′ segment of the small subunit ribosomal RNA (SSU) gene.

DNA sequences have the capability for species identification by acting as taxon barcodes or genetic barcodes which are embedded in every cells. Hebert *et al*. (2003a) established that the mitochondrial gene *cytochrome c oxidase I* (*COI*) can help global bioidentification system of animals. Significant limitations in species identification includes phenotypic plasticity and genetic variability in the characters employed for species recognition, morphological similarity between cryptic taxa and morphological keys effective only for a particular life stage or gender. Hebert *et al*. (2003b) identified different species of insects using mitochondrial *COI* gene and concludes that *COI* analysis can provide taxonomic system to chase animal species diversity.

According to Stoeckle (2003), DNA barcoding is a uniform, practical method for species identification, and also has broad scientific applications. It can be used for identification of eggs and larval forms, for instance, and analysis of stomach contents or excreta to determine food webs. He says that DNA barcoding of same genes from different organisms help to identify the species diversity. The reliability of DNA barcoding depends on reference sequences which are established from taxonomically confirmed specimens. The ideal gene for DNA barcoding should be conserved for amplifying with broad range of primers and also should be divergent enough to resolve closely related species. Since the mitochondrial genes of animals do not contain introns and also they share diverse taxa, they are the ideal target gene for DNA barcoding of animals. In case of plants, the mitochondrial DNA have little sequence variation due to hybridization and introgression. Chloroplast gene such as matK (maturase K) or a nuclear gene

such as *ITS* (*internal transcribed spacer*) may be an effective target for barcoding in plants.

According to Besansky *et al*. (2003), since traditional morphology-based assessments are time-consuming and require specialists whose numbers are insufficient and dwindling, DNA-based method called DNA barcoding can be used as a rapid means of cataloguing species. They also says that mitochondrial gene *cytochrome oxidase I* (*COI*) serves as the core of a global bioidentification system for animals

Barrett and Hebert (2005) identified spiders by DNA barcoding method using the sequence diversity of mitochondrial *COI* gene. The result of the study proved the efficacy of *COI* gene as rapid and accurate tool for identification of spiders. DNA barcoding using *COI* gene sequences aided species identification of lepidopteran insects in tropical areas (Hajibabaei *et al*., 2005). However Monaghan *et al.* (2005) suggested that DNA barcoding aims at identification of pre-defined species and it does not address the issue of species delineation itself.

Ball *et al*. (2005) identified 69 out of 70 mayfly species by DNA barcoding using *COI* sequences. Their results showed that *COI* sequences are effective in identifying mayflies from the northeastern United States and central Canada. Caesar *et al.* (2006) identified species diversity of edaphic beetles in the Klamath ecoregion, California, USA by integrating DNA sequence data of mitochondrial *COI* gene and traditional taxonomy. Mosquito species from Eastern Canada were identified by DNA barcoding, using the *COI* sequence variation (Cywinska *et al.*, 2006).

Smith *et al.* (2006) used DNA barcoding as a method for conforming the monophagous nature of Tachinidae insects using mitochondrial *COI* sequences. According to Smith, *COI* provides an attractive genetic barcode for species identification because of its high copy number, rapid rate of mutation, and ease of amplification/sequencing and alignment for intra- and interspecific comparisons.

According to Vogler and Monaghan (2007), DNA taxonomy differs from DNA barcoding by, the former directly concerns with the circumscription and delineation of species using evolutionary species concepts and the latter is a means of identifying a priori entities by sequence similarity. Dove *et al.* (2008) proved that DNA barcodes are useful for species identification of degraded samples such as those obtained from birdstrikes and *COI* termed 'the barcoding gene'.

The main aim of DNA barcoding is accurate species level identification and mitochondrial *COI* region is the standard barcode region for most of the animals (Packer *et al.,* 2009). DNA barcoding technique was effectively used for species identification of caterpillars collected from *Quercus rubra* and *Q. robur* trees from southern Germany (Gossner and Hausmann, 2009).

Research conducted by Lowenstein *et al.* (2009) demonstrated that tuna fish species could be reliably identified with *cox1* barcodes using either character-based key or highest BLAST sequence similarity. Valentini *et al.* (2009) demonstrated that DNA barcoding is fast, simple to implement, and very robust. It can be applied for diet analyses of a wide range of phytophagous species at large scales. They also demonstrated that this approach was efficient for mammals, birds, insects and molluscs.

According Hinomoto *et al.* (2009) mitochondorial *cytochrome oxidase subunit I* (*COI*) has been widely used in DNA barcoding technique; genes on mitochondrial DNA can be easily analysed, because of their maternal inheritance and monomorphism. Glover *et al*. (2010) compared five different loci *viz*., *COI*, *COII*, *COIII*, Histone H3 and *ITS*2 to inspect their ability in DNA barcoding of the genus *Thrips* to identify different species. Intraspecific and interspecific distances of different loci were examined and found that *COI* sequences provide enough variation for DNA barcoding of the genus *Thrips*.

DNA barcoding can be used as a powerful tool in conserving threatened organisms by preventing illegal trade. Reid *et al.* (2011) developed mitochondrial

COI sequences of 174 species of turtles and combined the data with publicly available data to represent the wideness of the order. Based on variation in barcode region and distance and character based methods, they identified the species.

According to Mutanen *et al.* (2012) DNA barcoding helps in revealing identity of cryptic species. Efforts to generate DNA barcodes for all North European Lepidoptera, *Phalonidia manniana* revealed that it belonged to two genetically distinct clusters *P. manniana* and *P. udana*. According to Taylor and Hariss (2012), the barcoding approach provides a framework for the survey of biodiversity - a crucial task for prioritising conservation efforts given the current extinction crisis.

Integrative taxonomy is the basis of DNA barcoding. Significant contribution of DNA barcoding is in conservation of known biodiversity. It enhances taxonomic research and conservation efforts but it cannot replace traditional taxonomic research (Krishnamurthy and Francis, 2012).

Analysis of *COI* sequences of species of mirids in India viz., *Helopeltis antonii* Signoret, *H. thievora* Waterhouse, *H. bradyi* Waterhouse, and *Pachypeltis maesarum* Kirkaldy showed less than one per cent intraspecific divergence for all four species, whereas interspecific divergence ranged from seven to 13 per cent. The study revealed the usefulness of DNA barcoding for the identification of mirids in India and as a decisive tool in quick identification of invasive and cryptic species (Rebijith *et al*., 2012).

Dona *et al*. (2015) DNA barcoded ectoparasitic feather mites of passerine birds from Russia and Spain using *COI* sequences to identify species. The DNA barcoding unravelled earlier taxonomic issues of the *Proctophyllodes pinnatus* group and three new cryptic species were identified.

Sadurudeen *et al*. (2017) generated DNA barcodes of 32 species of perciformes fishes belonging to thirteen families with *COI* sequences, among these ten species were new report from India into BOLD database. The genetic

divergence values increased from lower to higher taxa and the barcode gap analysis showed absence of overlapping between intra and interspecific divergence values.

DNA barcodes of 46 species of freshwater fishes covering the orders *Cypriniformes*, *Siluriformes*, *Synbranchiformes* and *Perciformes* representing 30 genera under 9 families were generated with *COI* sequences. The study indicated that *COI* can be used as the standard barcoding marker for identifying fish species (Raja and Perumal, 2017).

Study by Kumar *et al*. (2018) generated DNA barcode sequence of 44 Geometridae moths from Namdapha National Park in Eastern Himalaya. Among them, the DNA barcode data of 13 Geometridae species were the novel contribution to the global database, thus the study contributed DNA barcode data of taxonomically identified Geometridae species in the global database for succeeding research.

2.3. DNA barcoding of spider mites

It is very difficult to identify spider mites morphologically even by expert taxonomists because of its cryptic nature. For morphological identification, precisely slide mounted adult specimens are needed and it is cumbersome. The problem can be solved by molecular taxonomy.

Mitochondrial *COI* fragments were used to distinguish between four species of *Tetranychus* mites involved in quarantine problems associated with apple imports in North America (Lee and Lee, 1997). Navajas *et al*. (1998) revealed that in *T. urticae, cytochrome oxidase subunit I* (*COI*) on mitochondrial DNA is highly polymorphic within the species, and is therefore suitable for analyses of the intra-specific variation. Navajas (1998) conducted phylogenetic analysis with the mitochondrial *COI* sequences and it provided some clues concerning the colonization patterns of *T. urticae* in the regions sampled.

Hinomoto *et al.* (2001) revealed that although *T. urticae* belongs to two diverged lineages, the green and red forms of the mite, the two forms belong to

the same species by phylogenetic study using mitochondrial *COI* sequences. Hinomoto *et al*. (2007) classified most of the Vietnamese spider mite species into *Tetranychus kanzawai* Kishida, *T. urticae* Koch and *T. truncatus* Ehara using DNA barcoding technique. *COI* sequence data were useful for identifying *Tetranychus* species. They concluded that DNA barcoding along with phylogenetic analysis is an effective technique for species identification of spider mites.

Ros and Breeuwer (2007) analysed the phylogenetic tree of *COI* sequences of spider mites viz., *T. truncatus*, *T. urticae* and *T. kanzawai* and found that three of them formed different clades.

Based on the direction of curvature of aedeagus the genus *Oligonychus* is divided morphologically into two groups and also the genus includes some morphologically similar species that are difficult to distinguish. Matsuda *et al*. (2012) determined the species of *Oligonychus* by comparing the *COI*, *ITS* and 28S rDNA sequences for 17 species.

The identity of *Tetranychus truncatus*, which was recorded long years after the first report, was confirmed by comparing the *COI* and *ITS*2 sequences with the NCBI GenBank data base from the Asian region (Srinivasa *et al.*, 2012). The species identity of *Tetranychus turkestani* was also confirmed by using the *COI* sequences (Srinivasa *et al*., 2014).

Matsuda *et al.* (2013) examined the efficiency of *ITS*2 and *COI* sequences in species determination of *Tetranychus*. *ITS*2 and *COI* sequences of 13 known *Tetranychus* species in Japan were analysed by constructing phylogenetic tree. Ten of the 13 species were identified using *ITS*2 tree, while all the 13 species were identified using *COI* tree. The spider mite specis, *Tetranychus kanzawai* Kishida and *T. parakanzawai* Ehara were clearly separated into two monophyletic clades showed the existence of cryptic species in each species.

The studies conducted jointly by Centre for Plant Biotechnology and Molecular Biology and All India Network Project on Agricultural Acarology

in Kerala Agricultural University to document the diversity of spider mites infesting major crops of Kerala, using DNA barcoding technique had revealed that *COI* and *ITS*2 are potential markers for the technique (Bennur *et al*., 2015; Arunima, 2017).

Arabuli and Gotoh (2018) described a new species of spider mite, *Oligonychus neocastaneae* sp. nov. that closely resembled *Oligonychus castaneae* Ehara and Gotoh, 2007, and inhabited the same host plant, *Castanea crenata*, but mainly differed by the aedeagus. A maximum likelihood tree based on the *cytochrome c oxidase subunit I* (*COI*) gene of mitochondrial DNA (mtDNA) showed that *O. neocastaneae* sp. nov. was clearly separated from *O. castaneae* and other related species.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants" was carried out at the Department of Plant Biotechnology and Acarology Laboratory of All India Network Project on Agricultural Acarology (AINPAA), College of Horticulture during the period of 2017- 2019. The objectives of the study were to generate DNA barcodes for different species of spider mites infesting commercial ornamental plants of central Kerala and to find out the variability among them.

The materials and methods adopted for the conduct of the study are detailed in this chapter.

3.1. Collection, culturing and preservation of spider mites

3.1.1. Field survey

Purposive sampling surveys were carried out in commercial ornamental nurseries and homestead gardens of Thrissur and Ernakulam districts covering eleven different locations during November, 2017 to May 2019 (Fig. 1). During the survey, the ornamental plants *viz*., rose, jasmine, marigold, chrysanthemum, carnation, balsam, cock's comb, *Gerbera*, coleus, desert rose (*Adenium obesum*), *Bauhinia*, Cairo morning glory, orchid (*Vanda* sp.), *Zinnia* and pinto peanut were examined for mite infestation (Plate 1). For collection of mites, the leaves showing speckling symptoms, typical to spider mite infestation were observed using magnifying hand lens, for the presence of mites (Plate 2). Mite infested leaves were detached from plants, put in polythene bags tied with rubber bands, labelled properly with details of place and date of collection and brought to the laboratory. Materials used for the collection of mites are presented in the Annexure I.

3.1.2. Maintenance of isoline cultures

In the laboratory, the mite infested leaf samples were observed under stereo binocular microscope for the presence of spider mites. A single gravid

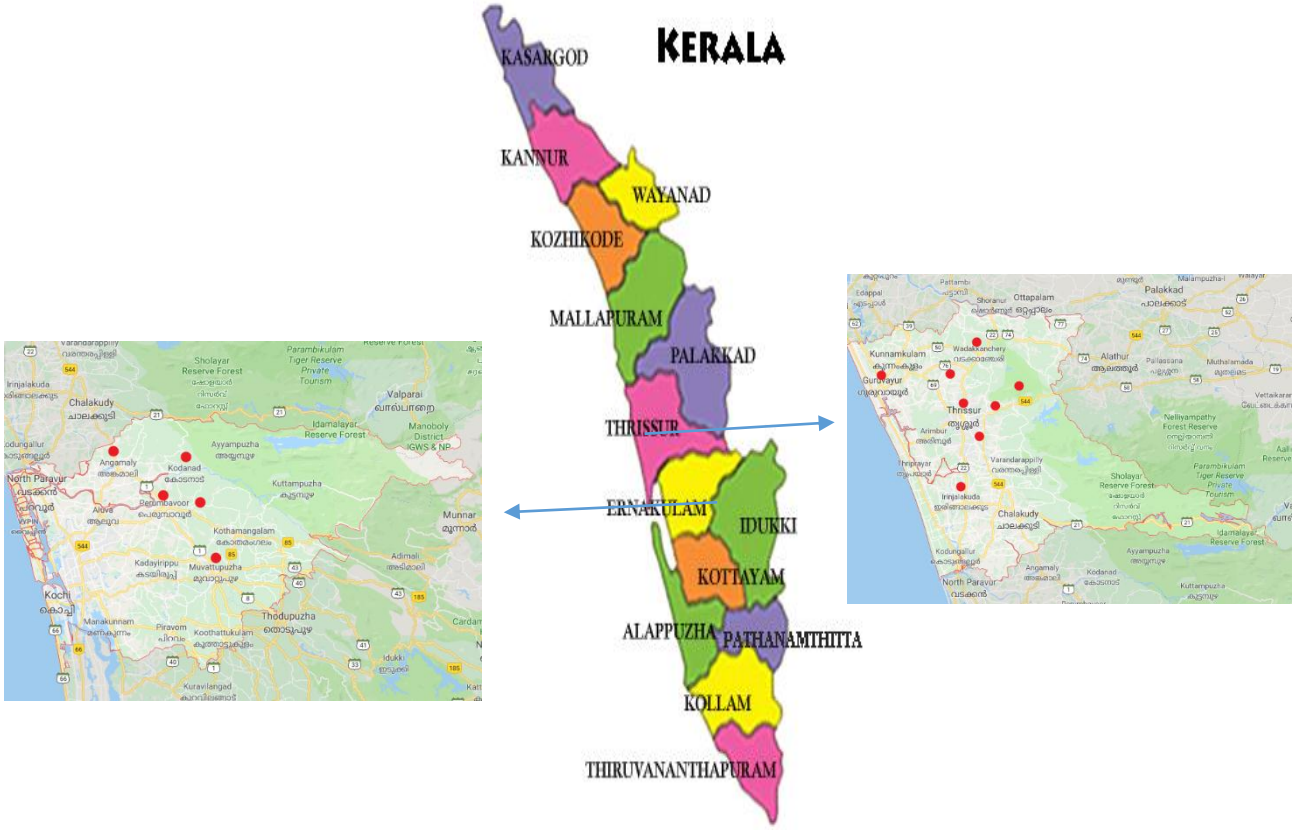


Fig. 1. Survey locations in Thrissur and Ernakulam districts

Plate 1. Survey conducted at various locations

A. Madakkathara B. Vellanikkara C. Mannuthy D. Odakkali

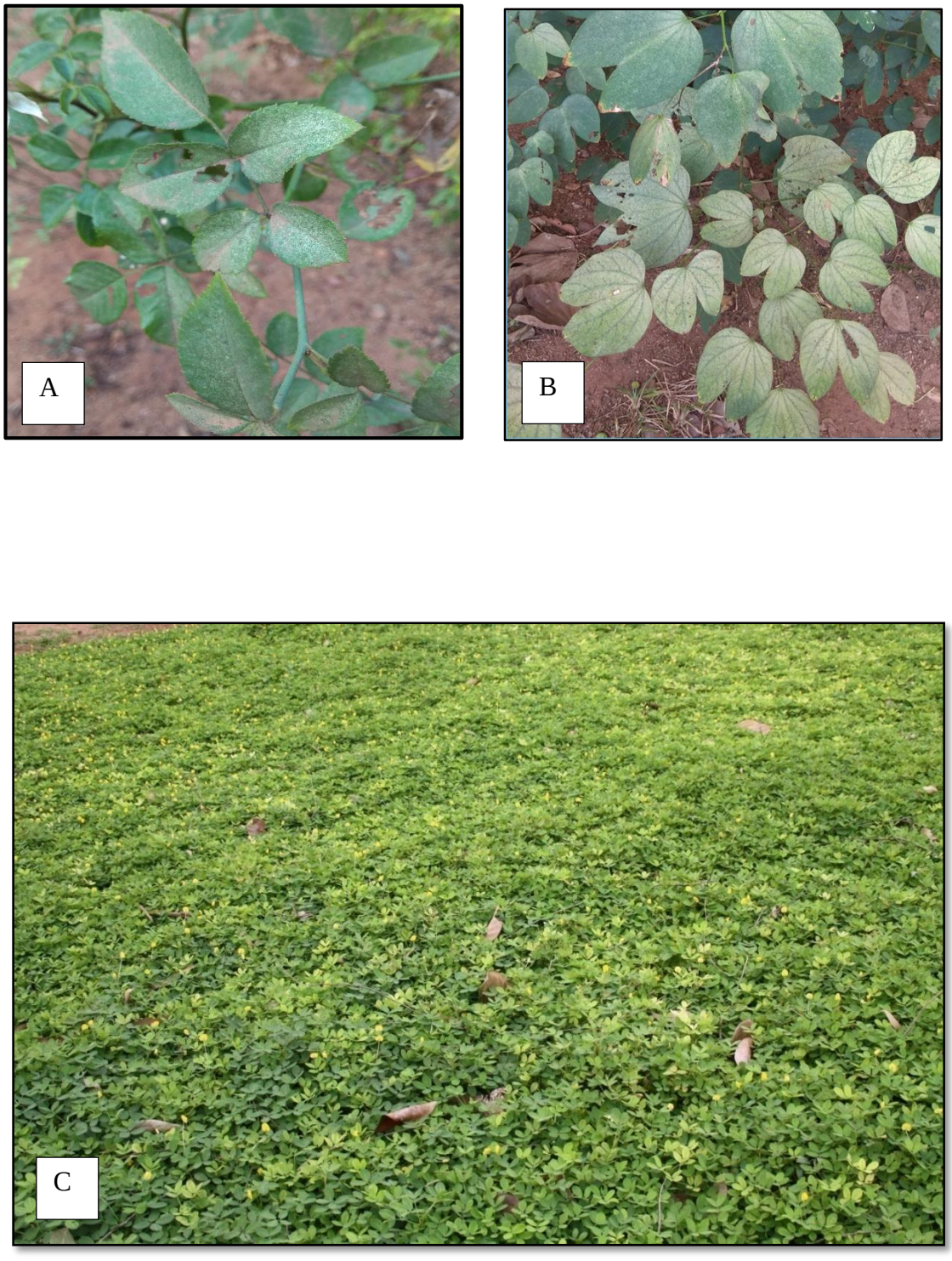


Plate 2. Symptoms of spider mite infestation

A. Rose B. *Bauhinia* C. Pinto peanut

female mite from each sample was transferred separately onto a fresh mulberry leaf, using fine camel hair brush. The leaf was then placed on a clean sponge surrounded by water, placed in a tray and maintained in the laboratory (Plate 3). The population arising from this single gravid female was maintained as isoline culture, assigning unique accession number. Isolines were maintained separately for mites collected from different plants, from different locations and on different dates.

3.1.3. Preservation of spider mites

3.1.3.1. Wet preservation

Adult male and female mite specimens from each isoline culture were picked separately and placed in 100 per cent alcohol in 0.5ml eppendorf tube and stored at 4 °C (Post *et al*., 1993).

3.1.3.2. Slide mounting of mite specimen

For morphological characterisation of mites, permanent slides of the male and female specimens were prepared from each isoline culture, separately. The specimen was mounted dorsally on a drop of Hoyer's medium, placed at the middle of the glass slide, pressed to the bottom of the medium droplet and arranged on a vertical axis. A cover slip was placed over the droplet without air bubbles for pressing the specimen. For observing the shape of aedeagus, a key character for species level identification, male mite was also mounted laterally (Henderson, 2001). The slides were labelled properly with the details of host, locality, date of collection and collector's name and then kept in hot air oven at 43 °C for one week. Heat treated slides from oven were taken outside and kept at room temperature for one day followed by ringing around the cover slip using a non-soluble sealant (Plate 4). Materials and equipment required for preparation of permanent slides are listed in Annexure I.

Plate 3. Isoline cultures of spider mites maintained in the Acarology laboratory

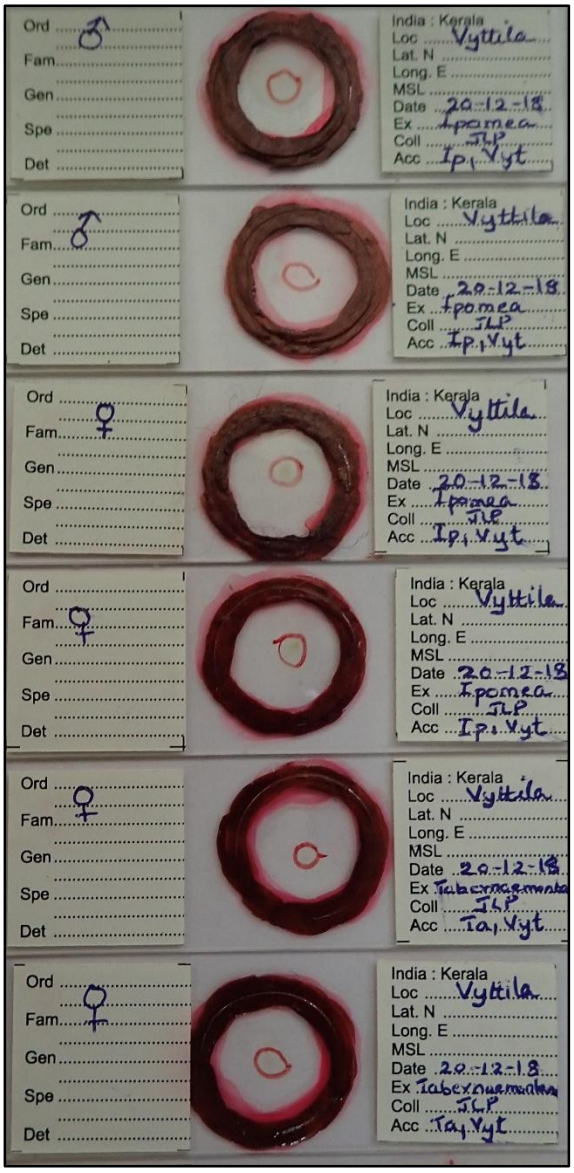


Plate 4. Permanent slides of male and female specimens of spider mite

3.2. Morphological characterisation and identification of mite specimens

The permanent slides of female and male mite specimens were observed under Leica DM 500 phase contrast microscope for morphological characterization. The morphological features *viz.*, chaetotaxy of hysterosoma and leg and structure of empodium of the female mite were studied for genus level identification. For species level identification, structure of aedeagus (male genitalia) was studied. Based on the morphological characters studied, species level identification was confirmed using standard taxonomic keys (Gupta, 1985; Gupta and Gupta, 1994; Ehara, 1995; Srinivasa *et al*., 2012)

3.3. Molecular identification of mite specimens

3.3.1. Isolation of genomic DNA

Isolation of DNA from mite specimens was done using modified CTAB method (Arunima, 2017) The reagents used for the preparation of CTAB buffer is furnished in Annexure II. Ten to fifteen adult female mites each were picked from isoline culture using a fine needle and placed in 20 µl CTAB buffer taken in a 1.5 ml eppendorf tube. The mites were crushed using micropestle and 80 µl of pre-warmed CTAB buffer was then added. The tube was kept for incubation at 60 °C for one hour in dry bath. After one hour, 100 µl of freshly prepared pre cooled chloroform: isoamyl alcohol (24:1) mixture was added and mixed well by inverting for two minutes. The mixture was then centrifuged at 10,000 rpm for 15 minutes at 4 °C. After centrifugation, three layers were formed and the topmost aqueous layer was transferred to a new pre chilled eppendorf tube with proper label. To this aqueous solution, 200 µl of ice cold 96 per cent ethanol and 30 µl of sodium acetate buffer were added. The mixture was kept for incubation at -20 °C overnight. On the next day, the mixture was centrifuged at 13,000 rpm for 10 minutes at 4°C. The supernatant was discarded and the pellet was washed with 100 µl ice cold 70 per cent and 100 per cent ethanol and centrifuged at 13,000 rpm for 10 minutes at 4 °C. After centrifugation, the supernatant was discarded and the pellet was dried at room temperature. After drying, the pellet was suspended in

10 µl double autoclaved water and stored at -20 °C. Reagents required for the preparation of buffers are listed in Annexure II.

3.3.2. Quantification of DNA using NanoDrop ND1000 spectrophotometer

The quality and quantity of the DNA isolated from different accessions of spider mites was analysed using NanoDrop spectrophotometer (ND-1000). Bases in nucleic acids showed absorption maxima around 260 nm if DNA is pure and proteins show peak absorbance at 280 nm. Absorbance was recorded at two wavelengths and purity was indicated by the ratio A260/A280. Absorbance ratio between 1.8 and 2.0 showed that the DNA preparation is pure and free from proteins. Value above 2.0 indicated RNA contamination, while value below 1.8 indicated protein contamination. ND -1000 software was used for assessing the quality and quantity. The procedure for analysis is given.

- NanoDrop spectrophotometer was connected to the computer system and the operating software ND-1000 was opened
- The option 'Nucleic acid' was selected in the programme
- One µl distilled water was placed onto the lower measurement pedestal, the sampling arm was lowered and the reading was set to zero on selecting the option 'Blank'
- After wiping the arms with tissue paper, 1µl of sample was pipetted into the measurement pedestal and the option 'measure' was selected and the reading was recorded
- After measurement, sampling arm was opened and the sample was wiped from both the upper and lower pedestals
- All the samples were loaded one after the other and readings were recorded following the same procedure

3.3.3. DNA amplification with Polymerase Chain Reaction (PCR)

Polymerase chain reaction process requires appropriate measures of components needed for the reaction mixture. Template DNA, Taq assay buffer,

dNTP, $MgCl_2$, forward and reverse primers (Table 1), Taq DNA polymerase enzyme and sufficient amount of water are the components of PCR. Reaction mixture was dispensed into 0.2 ml PCR tubes along with template DNA and subjected to thermal cycling. The candidate loci used for PCR analysis was C*ytochrome oxidase subunit I*. PCR amplification was done using Applied Biosystems Veriti 96-well thermal cycler.

For PCR amplification of *COI* locus, 25 µl of reaction mixture was prepared. The composition of the reaction mixture is given below (Li *et al*., 2010).

- Genomic DNA - 4 µl
- 10 X Taq assay buffer A - 2 µl
- dNTP mix (10 Mm) - 2 µl
- $MgCl_2$ - 0.75 µl
- Taq DNA polymerase - 0.5 µl
- Forward primer - 1.0 µl
- Reverse primer - 1.0 µl
- Autoclaved distilled water - 13.75 µl

Total volume - 25.0 µl

Thermal programme used for the amplification of *COI* locus is given below:

Initial denaturation	- 94 °C for 3 minute	
Denaturation	- 94 °C for 1 minute	35 cycles
COI primer annealing	- 59 °C for 1minute 30 seconds	
Primer extension	- 72 °C for 1 minutes 30 seconds	
Final extension	- 72 °C for 10 minutes	

The product was maintained at 4 °C till used for electrophoresis

Table 1. Details of primers used in the study

Sl. No.	Locus	Sequence (5′ – 3′)	Registered name of primer in BOLD	Reference
1	COI F	GGAGGATTTGGAAATTGATTAGTTCC	UBC6 F	Simon *et al.*, 1994
	COI R	GATAAAACGTAATGAAAATGAGCTAC	R COI	Gotoh *et al.*, 2009

3.3.4. Assessing the PCR products

The amplification of the *COI* locus of spider mite DNA was assessed by agarose gel electrophoresis on 1.2 per cent agarose gel.

- Agarose gel (1.2%) was prepared by boiling 1.2 g agarose in 100 ml 1X TAE buffer
- The solution was allowed to cool to about 42 °C to 45 °C. Ethidium bromide was added at this point
- Gel tray was prepared by sealing the ends and comb was placed in gel tray about 1 inch from one end of the tray and positioned vertically so that the teeth were about 1 to 2 mm above the surface of the tray
- Warm gel was poured into the tray to a depth of about 5mm and the gel was kept to solidify for 30 to 45 minutes at room temperature
- After solidifying, comb was removed and tray placed in electrophoresis chamber and filled with 1X TAE buffer
- Samples for loading were prepared by mixing 4 µl of 6X gel loading dye with 10 µl of PCR product and loaded in the wells. First well was loaded with 100 bp ladder

- Electrophoresis was carried out at 80 volts after connecting cathode and anode properly to the electrophoresis chamber. Electrophoresis continued until the dye moved to two thirds of the gel

The composition of buffers and dye for gel electrophoresis is listed in Annexure III.

3.3.5. Gel documentation

Electrophoresed gel documentation was done with BioRad gel documentation system. Quantity one software was used for imaging, analysing, and data basing the electrophoresed gel. The gel was exposed to UV radiations and the DNA present in the gel was expressed as bands of bright orange colour on the computer screen due to the intercalating ethidium bromide. The image was freezed at appropriate time and saved in JPEG format.

3.3.6. Sequencing of PCR product

The PCR products having single intact band were sequenced on Sanger platform at AgriGenome Labs, Pvt. Ltd., Cochin. The PCR products of twelve accessions were sequenced.

3.4. Data analysis using *in- silico* tools

3.4.1. Sequence annotation

The forward and reverse *COI* sequences of each accession were merged to form contigs using CAP3 sequence assembly programme.

3.4.2. Sequence homology analysis

Sequence homology of the contigs were assessed using Basic Local Alignment Seach Tool (BLAST), a sequence similarity search tool provided by National Centre for Bioechnology Information (NCBI). The individual sequences were uploaded in NCBI for nucleotide BLAST (BLASTn). The sequences in the database showing best E-value, maximum sequence identity percentage, query

coverage and least expected value with the sequences of accessions submitted were identified, based on which species were determined.

3.4.3. Barcode gap analysis

The 12 sequences generated in the present study along with two sequences of *Tetranychus okinawanus* Ehara and a reference sequence of *Tetranychus truncatus* Ehara retrieved from NCBI database were aligned using Clustal Omega tool. The barcode gaps among the different sequences representing various accessions were identified by recording the difference in the sequences at the region where '*' symbol is absent.

3.4.4. Calculation of pairwise distance

Pairwise distance of the sequences were analysed using MEGA X software. The aligned sequence was opened in MEGA X and pairwise distance was calculated using Kimura 2 Parameter model.

3.4.5. Construction of phylogenetic tree

A phylogenetic tree was constructed using "phylogeny" tool in MEGA X. Mitochondrial *COI* sequences of 12 accessions generated in the study along with 11 *COI* sequences of spider mites retrieved from NCBI database *viz*., *T. truncatus* (4), *T. okinawanus* (2), *O. biharensis* (2), *T. neocaledonicus* (1), *T. urticae* (1) and *T. puerariicola* (1) were used for the construction of the tree.

3.4.6. Submission to Barcode of Life Data Systems

DNA sequences (*COI*) of spider mites identified upto species level in the study (12 sequences) were submitted to Barcode of Life Data Systems (BOLD) online for generating species specific barcodes. Along with sequences, the specimen data, sequence data, traces, images and primer information were provided.


Results

4. RESULTS

The results of the study entitled 'DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants' carried out at the Department of Plant Biotechnology and All India Network Project on Agricultural Acarology, College of Horticulture, Vellanikkara during the period 2017- 2019 are detailed below.

4.1. Collection, culturing and preservation of spider mites

Purposive sampling surveys were conducted at Vellanikkara, Madakkathara, Mannuthy, Aryampadam, Paravattani, Manaloor, Elanadu, Guruvayur and Wadakkanchery in Thrissur district and Odakkali and Vyttila in Ernakulam district. Spider mites associated with ornamental plants *viz*., rose, marigold, chrysanthemum, balsam, cock's comb, *Gerbera*, *Adenium*, cairo morning glory, orchid (*Vanda* sp.), *Zinnia*, *Bauhinia* and pinto peanut were collected. Isoline cultures of the field collected spider mites were maintained in the Acarology laboratory, by assigning unique accession numbers. A total of 26 accessions of spider mites were maintained in the laboratory. Details of the accessions are given in Table 2.

4.2. Morphological characterisation and identification

Morphological characterisation of slide mounted specimens **(**male and female) representing 26 accessions maintained in the study revealed that six species of spider mites were associated with 12 ornamental plants surveyed in Thrissur and Ernakulam districts. The mite species belonged to two genera *viz*., *Tetranychus* Dufour and *Oligonychus* Berlese.

The genus *Tetranychus* was found to be more diverse representing five species *viz*., *Tetranychus truncatus* Ehara, *Tetranychus urticae* Koch, *Tetranychus okinawanus* Ehara, *Tetranychus neocaledonicus* Andre and *Tetranychus marianae* McGregor. The genus *Oligonychus* was represented by only one species, *Oligonychus biharensis* Hirst.

Table 2. Localities and ornamental plants surveyed for mite collection

Sl. no.	Accessions	Ornamental plants	Location	District	GPS co-ordinates		Date of collection
					Longitude	Latitude	
1	Ros1vk	Rose	Vellanikkara	Thrissur	10°54’64.7”N	76°27’60.4”E	21-09-2018
2	Ros2vk	Rose	Vellanikkara	Thrissur	10°32’34.0”N	76°16’34.8”E	20-02-2018
3	Ros3ap	Rose	Aryampadam	Thrissur	10°54’78.5”N	76°28’26.5”E	26-03-2019
4	Ros4mk	Rose	Madakkathara	Thrissur	10°33’10.3”N	76°15’29.9”E	13-04-2019
5	Mg1vk	Marigold	Vellanikkara	Thrissur	10°32’52.7”N	76°16’58.5”E	21-09-2018
6	Mg2vk	Marigold	Vellanikkara	Thrissur	10°32’53.6”N	76°16’57.0”E	08-12-2018
7	Cel1vk	Cock’s comb	Vellanikkara	Thrissur	10°32’15.7”N	76°16’37.8”E	18-12-2017
8	Cel2vk	Cock’s comb	Vellanikkara	Thrssur	10°32’52.2”N	76°16’58.1”E	21-09-2018
9	Chr2mk	Chrysanthemum	Madakkathara	Thrissur	10°54’74.8”N	76°28’38.3”E	12-04-2019
10	Zin1vk	*Zinnia*	Vellanikkara	Thrissur	10°32’34.0”N	76°16’34.8”E	20-02-2018
11	Arpin1od	Pinto peanut	Odakkali	Ernakulam	10°32’52.7”N	76°16’58.5’E	17-09-2018
12	Ger1vk	*Gerbera*	Vellanikkara	Thrissur	10°54’69.0”N	76°26’79.5”E	23-01-2018
13	Ros5vk	Rose	Vellanikkara	Thrissur	10°32’55.5”N	76°16’55.4”E	13-01-2018

…Table 2. Localities and ornamental plants surveyed for mite collection

Sl. no.	Accessions	Ornamental plants	Location	District	GPS co-ordinates		Date of collection
					Longitude	Latitude	
14	Ger2pv	*Gerbera*	Paravattani	Thrissur	10°52’05”N	76°24’36”E	18-01-2018
15	Ade1mn	*Adenium*	Manaloor	Thrissur	10°48’56.3”N	76°10’01.0”E	06-06-2018
16	Ade2vk	*Adenium*	Vellanikkara	Thrissur	10°54’74.4”N	76°28’23.7”E	13-07-2018
17	Arpin2vk	Pinto peanut	Veanikkara	Thrissur	10°32’52.7”N	76°16’58.5”E	17-09-2018
18	Bau1vk	*Bauhinia*	Vellanikkara	Thrissur	10°32’52.7”N	76°16’58.5”E	21-09-2018
19	Ipo1vyt	Cairo morning glory	Vyttila	Ernakulam	9°97’65.6”N	76°32’17.7”E	20-12-2018
20	Cel3ap	Cock’s comb	Aryampadam	Thrissur	10°54’78.0”N	76°28’26.5”E	26-03-2019
21	Bal1vk	Balsam	Vellanikkara	Thrissur	10°54’73.7”N	76°27’97.1”E	31-03-2019
22	Chr1vk	Chrysanthemu	Vellanikkara	Thrissur	10°54’74.8”N	76°28’38.3”E	12-04-2019
23	Orc1vk	Orchid	Vellanikkara	Thrissur	10°54’74.4”N	76°28’23.7”E	18-04-2019
24	Bal2wd	Balsam	Wadakkanchery	Thrissur	10°63’66.8”N	76°22’54.4”E	18-04-2019
25	Ros6en	Rose	Elanadu	Thrissur	10°61’64.7”N	76°38’75.5”E	19-04-2019
26	Mg3od	Marigold	Odakkali	Ernakulam	10°05’34.0”N	76°33’38.4”E	25-5-2019

Taxonomic key for the identification of spider mites associated with ornamental plants collected during the study is furnished below:

1a. Tarsus I with two sets of duplex setae distal and adjacent (Plate 5A); empodium of legs claw like with proximoventral hai (Plate 5B) ……..G**enus *Oligonychus* Berlese;** Aedeagus long and slender with axis of the knob parallel to the shaft; posterior projection of aedeagal knob acute with tip bending downward; dorsal surface of the knob nearly straight (Plate 7A)……. ……**.....*Oligonychus biharensis* Hirst**

1b. Tarsus I with two sets of duplex setae well separated (Plate 6A), dividing segment into three more or less equal parts; empodium of legs split distally into three pairs (Plate 6B)…**.....** **.....**……**...**Genus ***Tetranychus*** **Dufour** ……..……**...**……..**...2**

2a. Aedeagal knob with anterior projection rounded……**...**……..**3**

2b. Aedeagal knob with anterior projection not rounded……**...**……**4**

3a. Anterior projection of knob broadly rounded; posterior projectio of knob very narrow, acute resembling bird's beak (Plate 7B)……**...** ……..…***Tetranychus okinawanus*** **Ehara**

3b. Anterior and posterior projection of knob rounded and berry like; anterior projection better developed than posterior projection (Plate 7C)……**...** ….……....***Tetranychus neocaledonicus*** **Andre**

4a. Dorsum of aedeagal knob convex; anterior and posterior projections acute (Plate 7D)…….**....*Tetranychus urticae*** **Koch**

4b. Dorsal surface of aedeagal knob not convex; anterior projection of knob

not acute..……...……**5**

5a. Dorsal margin of the knob with a medial indentation near the posterior half (Plate 7E)…….....***Tetranychus truncatus*** **Ehara**

5b. Dorsal margin of the aedeagal knob without indentation**,** knob with a long posterior angulation (Plate 7F)……...

…...……..***Tetranychus marianae*** **McGregor**

4.3. Spider mites associated with ornamental plants

4.3.1. Diversity of spider mites

The diversity of spider mites associated with ornamental plants surveyed during the study is furnished in Table 3. The diversity was found to be maximum on rose plant which recorded five species *viz*., *T. truncatus*, *T. urticae*, *T. okinawanus*, *T. marianae* and *O. biharensis*, from five different localities surveyed. Both *Gerbera* and chrysanthemum recorded two species *viz*., *T. okinawanus* and *T. urticae*, while marigold recorded *T. okinawanus* and *T. truncatus*. All other plants recorded only one species. *Tetranychus okinawanus* was found on *Adenium* and balsam, while *O. biharensis* was found to be associated with *Bauhinia* and pinto peanut. *Zinnia*, cock's comb and cairo morning glory recorded *T. neocaledonicus*, *T. truncatus* and *T. okinawanus*, respectively.

4.3.2. Host range of spider mites

The spider mite species, *T. okinawanus* recorded wider host range and the associated host plants include rose, *Gerbera*, *Adenium*, balsam, marigold, chrysanthemum, orchid and cairo morning glory. The species, *T. truncatus, T. urticae* and *O. biharensis* recorded three host plants each. Rose, cock's comb and marigold were recorded as host plants of *T. truncatus*. *Tetranychus urticae* was recorded on rose, *Gerbera* and chrysanthemum, while *O. biharensis* was recorded on rose, pinto peanut and Bauhinia. *Tetranychus neocaledonicus* was associated

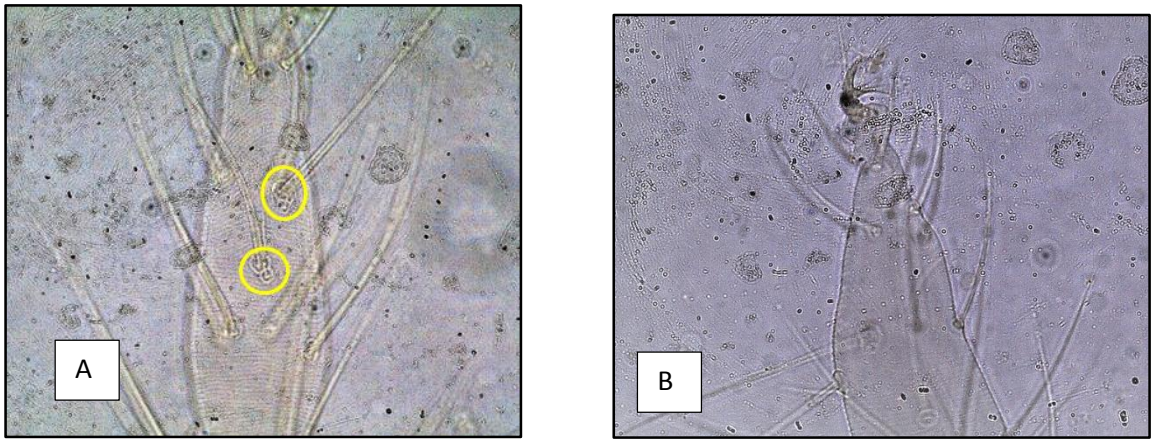


Plate 5. Key characters of the genus *Oligonychus*
A. Duplex setae B. Empodium (100 X)

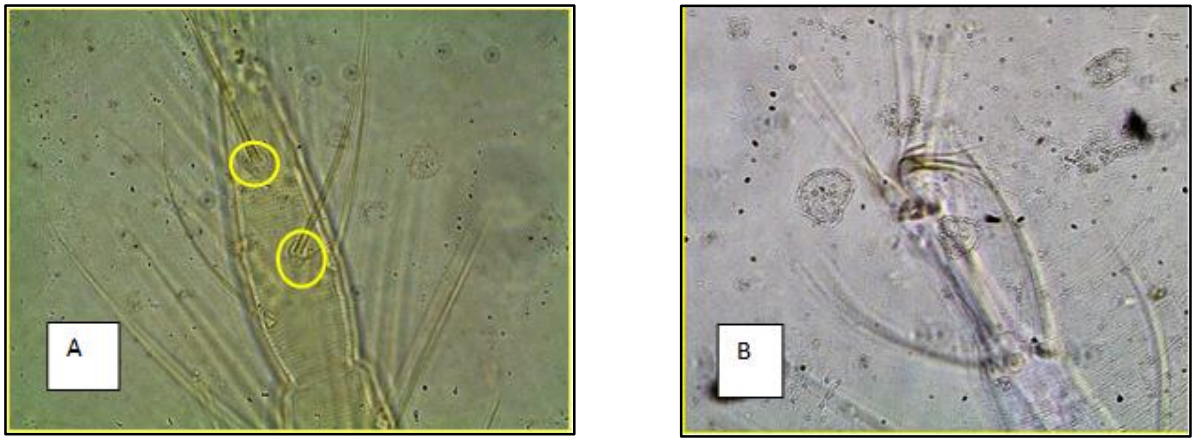


Plate 6. Key characters of the genus *Tetranychus*
A. Duplex setae B. Empodium (100 X)

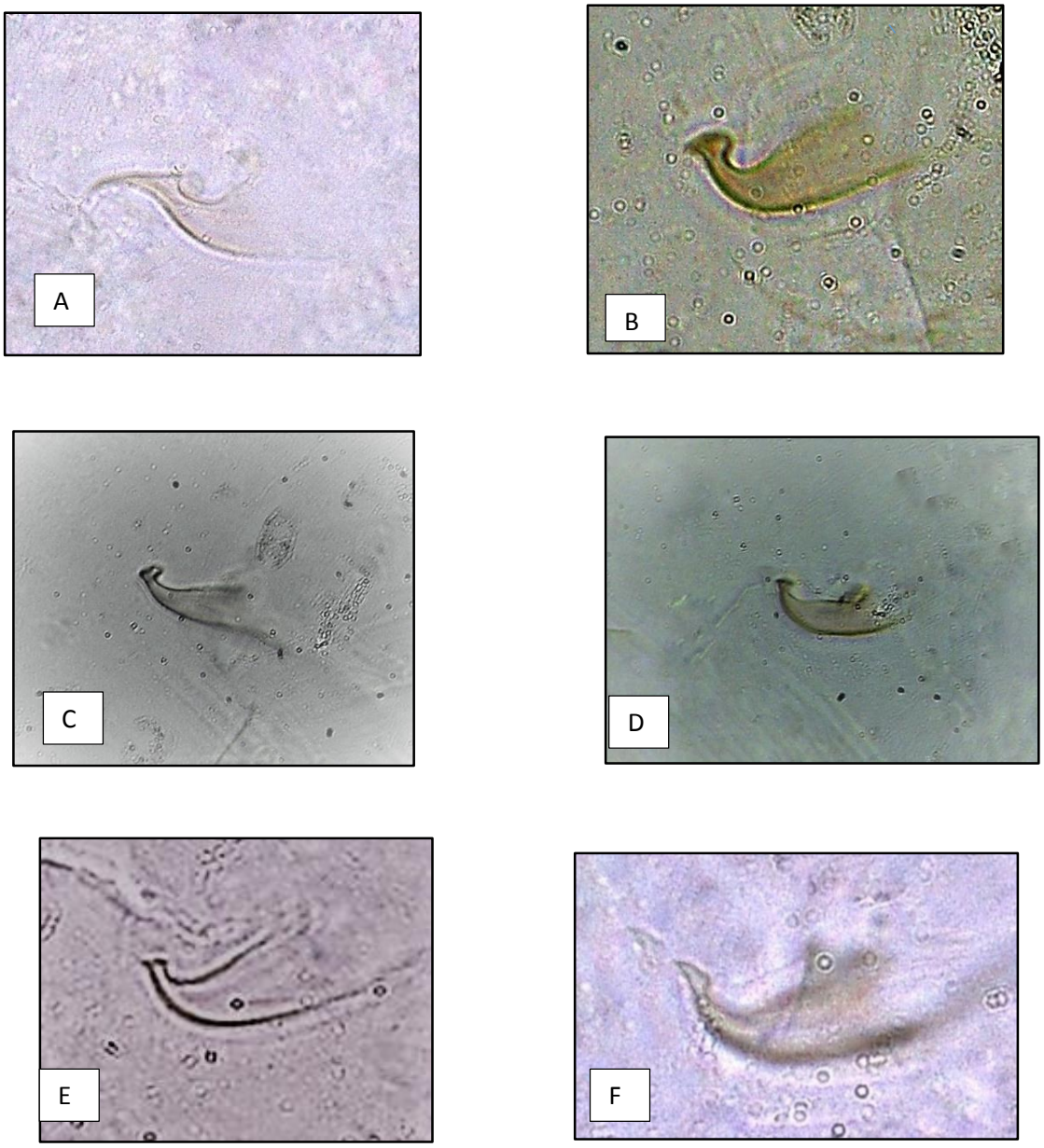


Plate 7. Aedeagus of species of *Oligonychus* and *Tetranychus* (100 X)

A. *O. biharensis* **B. *T. okinawanus*** **C. *T. neocaledonicus***
D. *T. urticae* **E. *T. truncatus*** **F. *T. marianae***

Table 3. Diversity of spider mites on ornamental plants

Sl. No.	Crop	Location	Species
1	Rose	Vellanikkara, Thrissur	*Tetranychus okinawanus* Ehara
		Vellanikkara, Thrissur	*Tetranychus urticae* Koch
		Vellanikkara, Thrissur	*Tetranychus marianae* McGregor
		Elanadu, Thrissur	*Tetranychus truncatus* Ehara
		Aryampadam, Thrissur	*Oligonychus biharensis* Hirst
2	*Gerbera*	Vellanikkara, Thrissur	*Tetranychus urticae* Koch
		Paravattani, Thrissur	*Tetranychus okinawanus* Ehara
3	Chysanthemum	Vellanikkra, Thrissur	*Tetranychus okinawanus* Ehara
		Madakkathara, Thrissur	*Tetranychus urticae* Koch
4	Marigold	Vellanikkara, Thrissur	*Tetranychus okinawanus* Ehara
		Vellanikkara, Thrissur	*Tetranychus truncatus* Ehara
5	*Adenium*	Manaloor, Thrissur	*Tetranychus okinawanus* Ehara
		Vellanikkara, Thrissur	
6	Cock's comb	Vellanikkara , Thrissur	*Tetranychus truncatus* Ehara
		Vellanikkara, Thrissur	
7	Pinto peanut	Vellanikkara, Thrissur	*Oligonychus biharensis* Hirst
		Odakkali, Ernakulam	
8	Balsam	Vellanikkara, Thrissur	*Tetranychus okinawanus* Ehara
		Wadakkanchery, Thrissur	
9	*Zinnia*	Vellanikkara, Thrissur	*Tetranychus neocaledonicus* Andre
10	*Bauhinia*	Vellanikkara, Thrissur	*Oligonychus biharensis* Hirst
11	Cairo morning glory	Vyttila, Thrissur	*Tetranychus okinawanus* Ehara
12	Orchid	Vellanikkara, Thrissur	*Tetranychus okinawanus* Ehara

with only *Zinnia*, while *Tetranychus marianae* was associated with only rose plant (Table 4).

4.4. Molecular characterisation

4.4.1. DNA isolation

Genomic DNA of spider mites of twelve accessions collected from different locations of Thrissur and Ernakulam districts were isolated using modified CTAB method (Arunima, 2017).

The quality and quantity of isolated DNA was checked by NanoDrop ND-1000 spectrophotometer. The $A_{260/280}$ ratio ranged between 1.93 to 2.14, while the quantity of DNA ranged from 100 to 402 ng/µl. The quality and quantity of DNA isolated from different accessions assessed by NanoDrop ND-1000 Spectrophotometer are furnished in Table 5.

4.4.2. Amplification of the barcode locus *COI*

The *COI* region of the spider mite DNA was amplified using universal primer, specific for spider mites reported by Li *et al.* (2010). The fragment length of amplified *COI* region of DNA was 800 - 900 bp. The quality of PCR products were checked by agarose gel electrophoresis using 1.2 per cent agarose. Bands at 800-900 bp length were obtained on agarose gel (Plate 8).

4.4.3. Sequencing of PCR products

The PCR products from all the 12 accessions, confirmed to have only one band on electrophoresis, were sequenced by outsourcing to AgriGenome Labs. Pvt. Ltd., Cochin. Sequence details are given in Annexure IV.

4.5. Analysis of molecular data using *in- silico* tools

4.5.1. Sequence homology analysis

Forward and reverse *COI* sequences were merged to form contigs using CAP3 sequence asssembler. Homology of the sequences were studied using nucleotide BLAST of NCBI database. The sequences showing maximum

Table 4. Host range of spider mites

Sl No.	Species	Host plant
1	*Tetranychus okinawanus* Ehara	Rose, chrysanthemum, *Gerbera*, marigold, *Adenium*, cairo morning glory, balsam, orchid
2	*Tetranychus truncatus* Ehara	Rose, cock's comb, marigold
3	*Tetranychus urticae* Koch	Rose, *Gerbera*, chrysanthemum
4	*Oligonychus biharensis* Hirst	Rose, pinto peanut, *Bauhinia*
5	*Tetranychus neocaledonicus* Andre	*Zinnia*
6	*Tetranychus marianae* McGregor	Rose

Table 5. Quality and quantity of genomic DNA of spider mites measured using NanoDrop ND1000 Spectrophotometer

Sl. No.	Accession number	$A_{260/280}$	Concentration (ng/ µl)
1	Mg1vk	2.14	128.0
2	Mg2vk	2.01	150.2
3	Arpin1od	2.13	202.9
4	Zin1vk	2.02	182.7
5	Ger1vk	2.03	187.3
6	Ros1vk	2.13	117.1
7	Ros2vk	2.11	171.5
8	Ros3ap	2.05	100.8
9	Ros4mk	1.98	121.9
10	Cel1vk	2.11	248.8
11	Cel2vk	1.93	402.6
12	Chr2mk	2.01	229.4

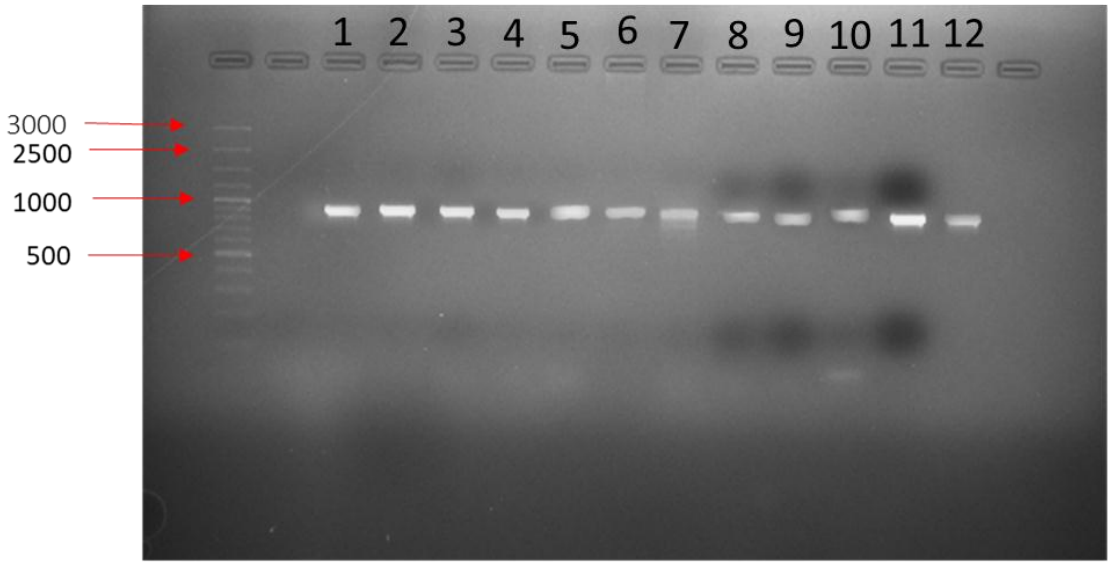


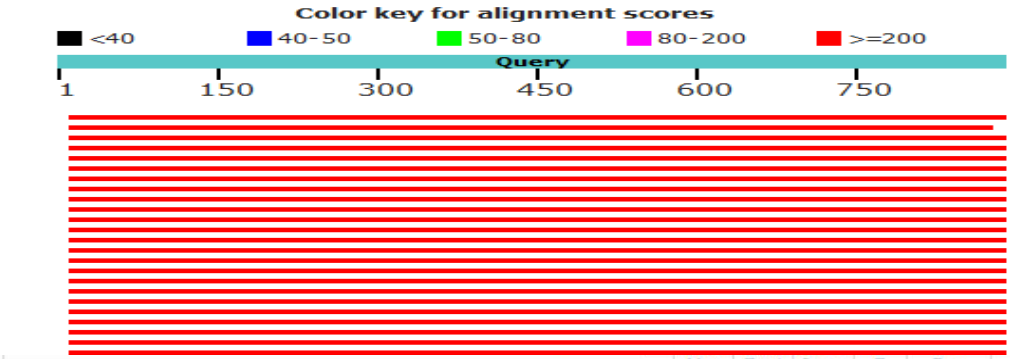
Plate 8. Amplification of *COI* locus of spider mites

M- ladder 1- Ros1vk 2- Cel1vk 3- Cel2vk 4- Mg1vk 5- Mg2vk 6- Chr2mk 7- Ros2vk 8- Ger1vk 9- Ros3ap 10- Ros4mk 11- Arpin1od 12- Zin1vk

similarity percentage, query coverage as well as E value of zero with those in the database were identified. The results of the BLAST analysis are presented in Figs. 2–13 and Table 6. Accordingly, the species identity of eleven accessions was established. The accessions, Cel1vk (Cock's comb, Vellanikkara) and Cel2vk (Cock's comb, Vellanikkara) showed maximum similarity with the NCBI accession KR072563 and KR052245, respectively, reported by Bennur *et al.* (2015) as *Tetranychus truncatus* Ehara. The accessions Mg1vk (Marigold, Vellanikkara) and Mg2vk (Marigold, Vellanikkara) showed maximum similarity with *Tetranychus truncatus* Ehara of the NCBI accessions, MF774629 and MF774634 reported by Arunima *et al*. (2017). The accessions Ros2vk (Rose, Vellanikkara), Ros4mk (Rose, Madakkathara), Ger1vk (*Gerbera*, Vellankkara) and Chr1vk (Chrysnthemum, Vellanikkara) showed maximum similarity with NCBI accession MK508722.1 reported by Inak *et al.* (2019) as *Tetranychus urticae* Koch. Though the accessions, Ros3ap (Rose, Aryapadam) and Arpin1od (pinto peanut, Odakkali) showed maximum similarity with *Oligonychus biharensis* Hirst (AB683679.1) reported by Matsuda *et al*. (2012), the similarity was 91.29 and 91.39 per cent, respectively. The accession Zin1vk (*Zinnia*, Vellanikkara) showed maximum similarity (94.95%) with the NCBI accession AB736057.1 reported by Mastuda *et al.* (2013) as *Tetranychus neocaledonicus* Andre. However, the accession Ros1vk did not show significant similarity with any of the *COI* sequences in the database. In the database, it had shown a similarity of only 87.97 per cent with the accession, MG518352 representing *Tetranychus puerariсola* Ehara and Gotoh.

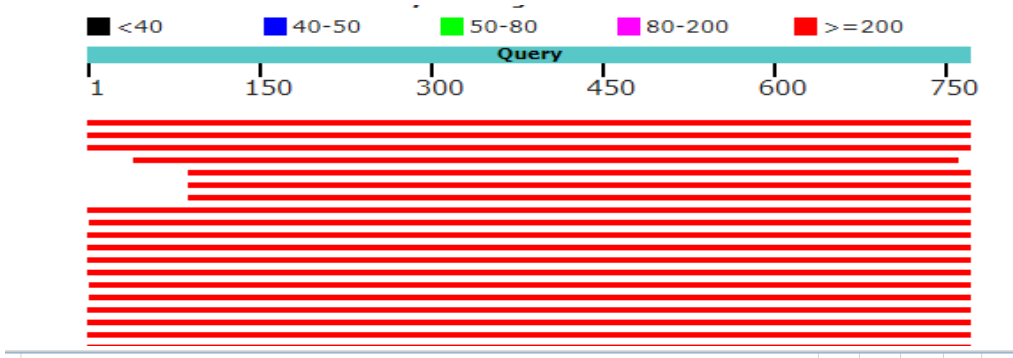
4.5.2. Barcode gap analysis

The twelve sequences generated in the present study, along with two sequences of *Tetranychus okinawanus* Ehara (AB736059 and AB736058) and a reference sequence of *Tetranychus truncatus* Ehara (KR072563) retrieved from NCBI database were aligned using Clustal Omega tool in MEGA X software. The maximum length of aligned sequences was 911 bp. The barcode gaps were



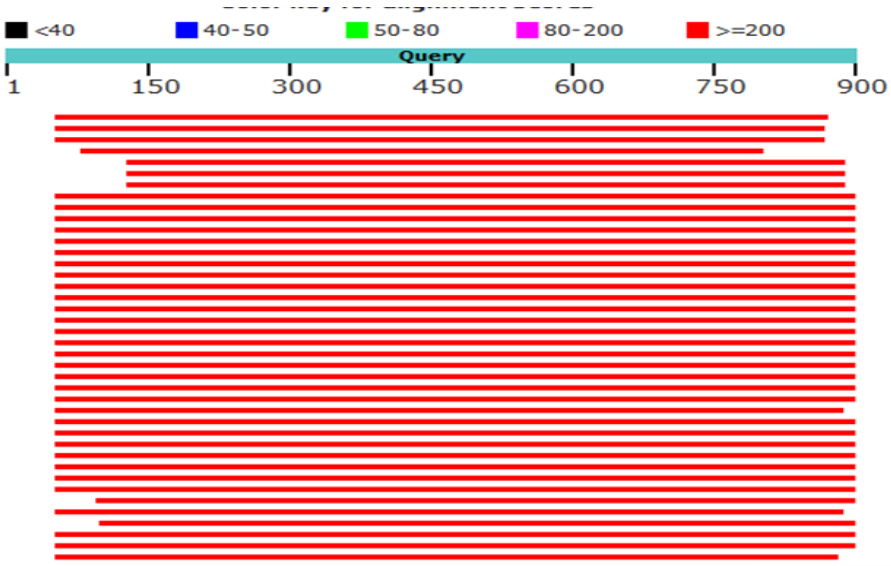
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus truncatus haplotype Tt17, mitochondrion, complete genome	1598	1598	98%	0.0	99.43%	MG518333.1
Tetranychus truncatus cytochrome oxidase subunit I gene, partial cds; mitochondrial	1598	1598	97%	0.0	99.88%	KR072563.1
Tetranychus truncatus haplotype Tt14 mitochondrion, complete genome	1592	1592	98%	0.0	99.32%	MG518343.1
Tetranychus truncatus haplotype Tt13 mitochondrion, complete genome	1592	1592	98%	0.0	99.32%	MG518342.1
Tetranychus truncatus haplotype Tt9 mitochondrion, complete genome	1592	1592	98%	0.0	99.32%	MG518341.1

Fig. 2. BLASTn result of accession Cel1vk



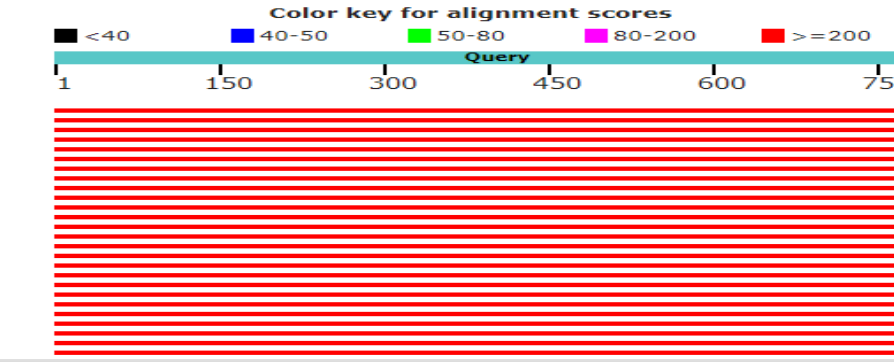
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus truncatus isolate OkEr12126 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1386	1386	100%	0.0	99.09%	MF774636.1
Tetranychus truncatus isolate TpCm1827 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1386	1386	100%	0.0	99.09%	MF774631.1
Tetranychus truncatus isolate Tpkk0227 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1386	1386	100%	0.0	99.09%	MF774629.1
Tetranychus udaipurensis cytochrome oxidase subunit 1 (CO1) gene, partial cds; mitochondrial	1312	1312	93%	0.0	99.58%	KU738616.1
Tetranychus neocaledonicus mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: Tne0008	1011	1011	88%	0.0	93.41%	AB736057.1

Fig. 3. BLASTn result of accession Cel2vk



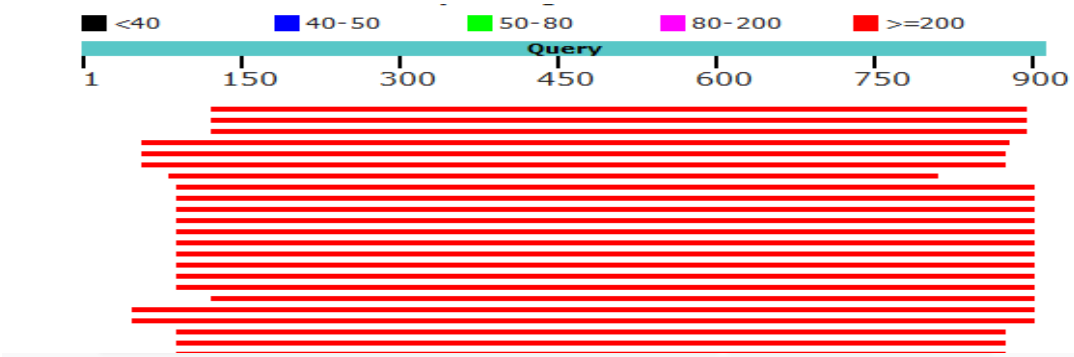
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus truncatus isolate Tpkk0227 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1448	1448	90%	0.0	98.66%	MF774629.1
Tetranychus truncatus isolate OkEr12126 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1439	1439	90%	0.0	98.65%	MF774636.1
Tetranychus truncatus isolate TpCm1827 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1439	1439	90%	0.0	98.65%	MF774631.1

Fig. 4. BLASTn result of accession Mg1vk



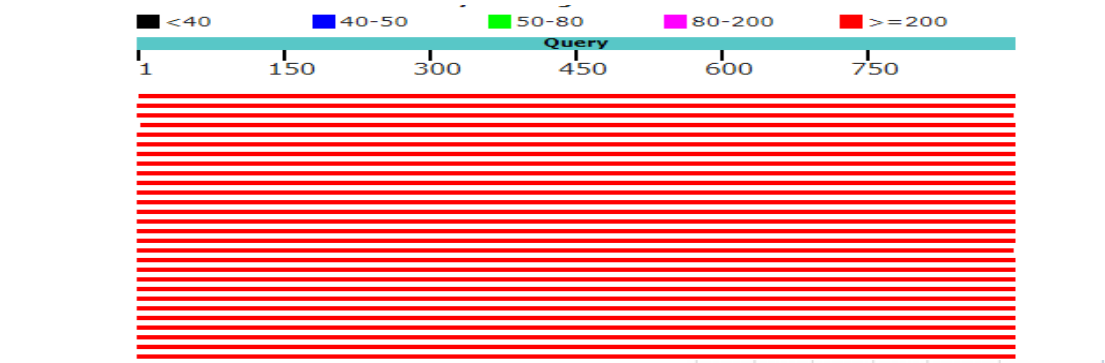
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus truncatus isolate BaKo1037 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1478	1478	100%	0.0	100.00%	MF774634.1
Tetranychus truncatus isolate CpVk19116 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1478	1478	100%	0.0	100.00%	MF774633.1
Tetranychus truncatus isolate TpOk12126 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1478	1478	100%	0.0	100.00%	MF774630.1
Tetranychus truncatus cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	1478	1478	100%	0.0	100.00%	KR733110.1
Tetranychus truncatus isolate VA1.2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1478	1478	100%	0.0	100.00%	KR052245.1

Fig. 5. BLASTn result of accession Mg2vk



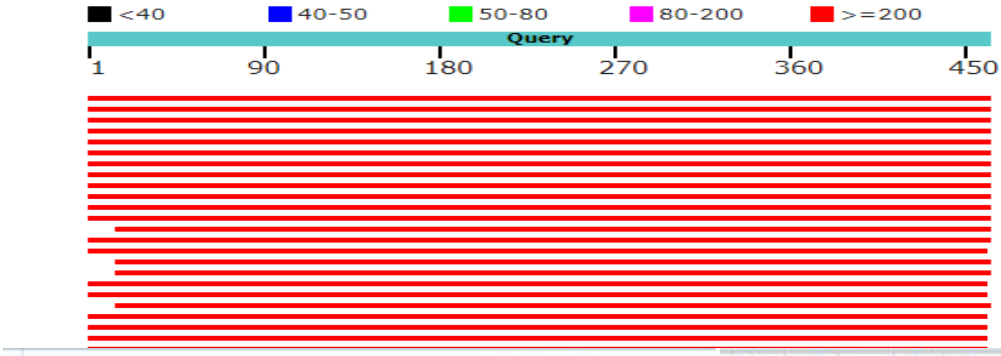
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus neocaledonicus mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: Tne0008	1195	1195	84%	0.0	94.95%	AB736057.1
Tetranychus neocaledonicus mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: Tne0003	1190	1190	84%	0.0	94.83%	AB736056.1
Tetranychus neocaledonicus mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: Tne0192	1190	1190	84%	0.0	94.83%	AB736055.1
Tetranychus truncatus isolate Tpkk0227 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1075	1075	89%	0.0	90.66%	MF774629.1
Tetranychus truncatus isolate OkEr12126 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1066	1066	89%	0.0	90.60%	MF774636.1
Tetranychus truncatus isolate TpCm1827 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1066	1066	89%	0.0	90.60%	MF774631.1

Fig. 6. BLASTn result of accession Zin1vk



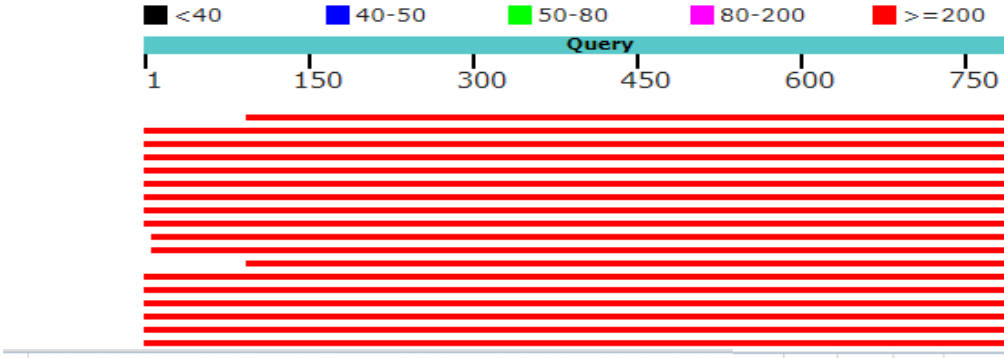
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus puerariicola haplotype TP10, mitochondrion, complete genome	1059	1059	99%	0.0	87.97%	MG518352.1
Tetranychus phaselus mitochondrion, complete genome	1059	1059	100%	0.0	87.92%	KJ729020.1
Tetranychus urticae strain 2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1051	1051	99%	0.0	87.85%	MK508713.1
Tetranychus truncatus cytochrome oxidase subunit I gene, partial cds; mitochondrial	1048	1048	99%	0.0	87.86%	KR072563.1
Tetranychus truncatus haplotype Tt29 mitochondrion, complete genome	1044	1044	100%	0.0	87.69%	MG518336.1

Fig. 7. BLASTn result of accession Ros1vk



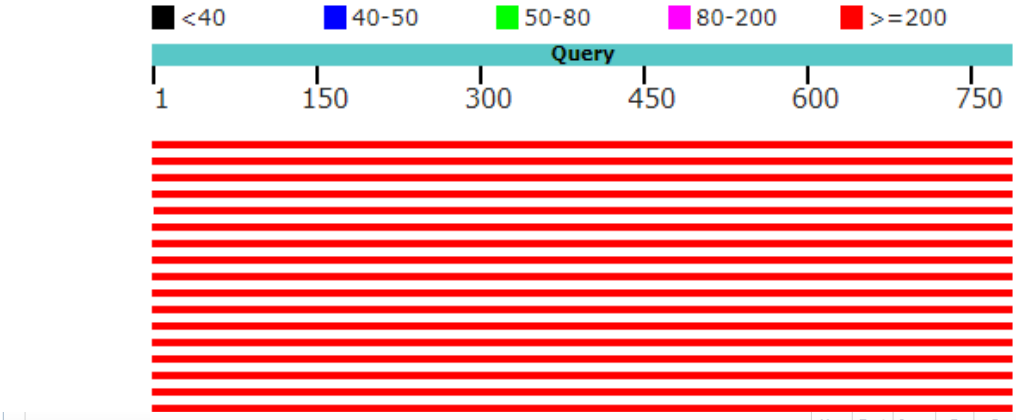
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
	Tetranychus urticae strain GSS cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	839	839	100%	0.0	99.57%	MK508722.1
	Tetranychus urticae strain 7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	839	839	100%	0.0	99.57%	MK508718.1
	Dioscorea rotundata mitochondrial DNA, contig: TDr_Mt_scaffold4_size13096_cultivar: TDr96_F1	839	839	100%	0.0	99.57%	LC219377.1
	Tetranychus urticae strain 13CB_JC_G1 cytochrome c oxidase subunit I (COXI) gene, partial cds; mitochondrial	839	839	100%	0.0	99.57%	KF977414.1
	Tetranychus urticae mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: TuG0185	839	839	100%	0.0	99.57%	AB736077.1

Fig. 8. BLASTn result of accession Ros2vk



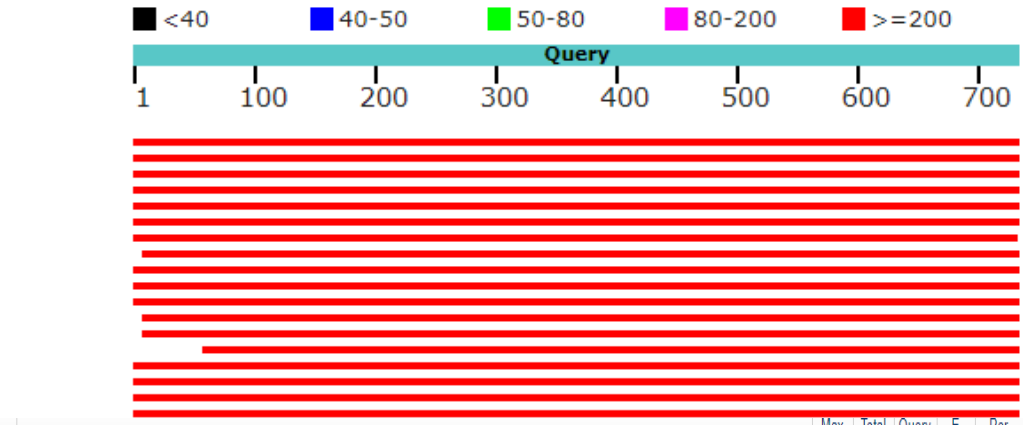
	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
	Oligonychus biharensis mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: OB0064	1002	1002	88%	0.0	91.29%	AB683679.1
	Tetranychus evansi strain Venancio Aires cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	985	985	100%	0.0	88.16%	GU145110.1
	Tetranychus evansi strain KM cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	985	985	100%	0.0	88.16%	FJ440678.1
	Tetranychus evansi strain TW cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	985	985	100%	0.0	88.16%	FJ440677.1
	Tetranychus evansi strain BP cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	985	985	100%	0.0	88.16%	FJ440675.1
	Tetranychus evansi strain North East Brazil cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	979	979	100%	0.0	88.04%	GU145111.1

Fig. 9. BLASTn result of accession Ros3ap



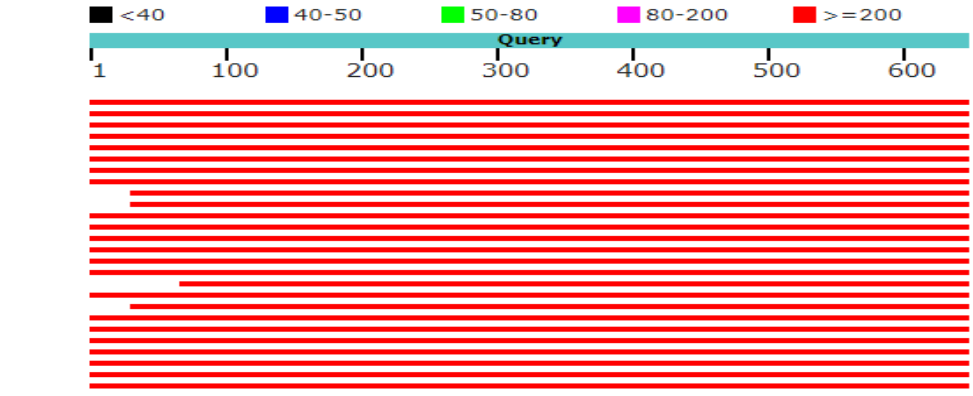
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus urticae strain GSS cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1424	1424	100%	0.0	99.49%	MK508722.1
Dioscorea rotundata mitochondrial DNA, contig: TDr_Mt_scaffold4_size13096, cultivar: TDr96_F1	1424	1424	100%	0.0	99.49%	LC219377.1
Tetranychus urticae strain 7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1419	1419	100%	0.0	99.36%	MK508718.1
Tetranychus urticae strain DeLier1 cytochrome oxidase subunit I gene, partial cds; mitochondrial	1413	1413	100%	0.0	99.24%	KF447574.1
Tetranychus urticae voucher UMMZ BMOC 07-0815-020 AD1014 cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	1411	1411	99%	0.0	99.23%	KY922440.1

Fig. 10. BLASTn result of accession Ros4mk



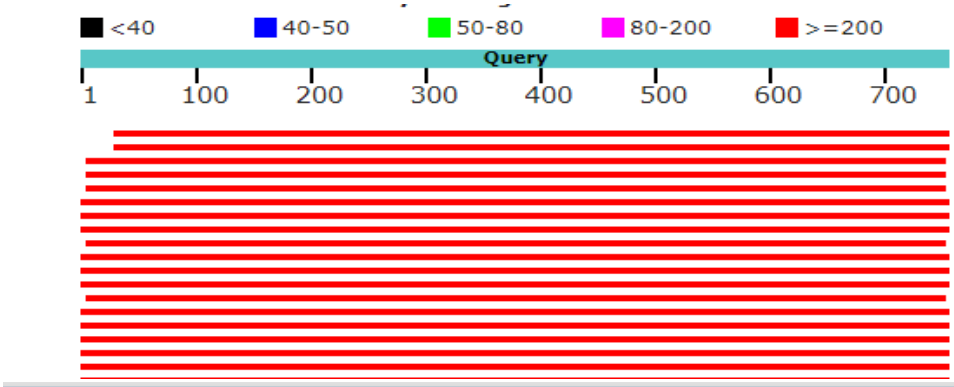
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus urticae strain GSS cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1327	1327	100%	0.0	99.32%	MK508722.1
Tetranychus urticae strain 7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1327	1327	100%	0.0	99.32%	MK508718.1
Dioscorea rotundata mitochondrial DNA, contig: TDr_Mt_scaffold4_size13096, cultivar: TDr96_F1	1327	1327	100%	0.0	99.32%	LC219377.1
Tetranychus urticae strain DeLier1 cytochrome oxidase subunit I gene, partial cds; mitochondrial	1327	1327	100%	0.0	99.32%	KF447574.1
Tetranychus urticae voucher UMMZ BMOC 07-0815-020 AD1014 cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	1323	1323	100%	0.0	99.18%	KY922440.1

Fig. 11. BLASTn result of accession Ger1vk



Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Tetranychus urticae strain GSS cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1140	1140	100%	0.0	98.61%	MK508722.1
Tetranychus urticae strain 7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1140	1140	100%	0.0	98.61%	MK508718.1
Dioscorea rotundata mitochondrial DNA, contig: TDr_Mt_scaffold4_size13096, cultivar: TDr96_F1	1140	1140	100%	0.0	98.61%	LC219377.1
Tetranychus urticae strain 13CB_JC_G1 cytochrome c oxidase subunit I (COXI) gene, partial cds; mitochondrial	1140	1140	100%	0.0	98.61%	KF977414.1
Tetranychus urticae voucher UMMZ BMOC 07-0815-020 AD1014 cytochrome oxidase subunit I (COXI) gene, partial cds; mitochondrial	1129	1129	100%	0.0	98.30%	KY922440.1

Fig. 12. BLASTn result of accession Chr2mk



Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Oligonychus biharensis mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: OB0064	985	985	96%	0.0	91.39%	AB683679.1
Oligonychus biharensis mitochondrial cox1 gene for cytochrome oxidase subunit I, partial cds, strain: OB0012	952	952	96%	0.0	90.56%	AB683678.1
Tetranychus evansi strain Vicosa1 cytochrome oxidase subunit I gene, partial cds; mitochondrial	918	918	98%	0.0	89.07%	KF447575.1
Tetranychus evansi strain Venancio Aires cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	918	918	98%	0.0	89.07%	GU145110.1

Fig. 13. BLASTn result of accession Arpin1od

Table 6. Homology of sequences of spider mite accessions in the study

Sl. No.	Accession	Mite species	Query coverage	Identity per cent (%)	E value
1	Cel1vk	*Tetranychus truncatus* Ehara	97	99.88	0.00
2	Cel2vk	*Tetranychus truncatus* Ehara	100	99.09	0.00
3	Mg1vk	*Tetranychus truncatus* Ehara	90	98.66	0.00
4	Mg2vk	*Tetranychus truncatus* Ehara	99	99.76	0.00
5	Zin1vk	*Tetranychus neocaledonicus Andre*	84	94.95	0.00
6	Ros1vk	*Tetranychus puerariсola* Ehara and Gotoh	99	87.97	0.00
7	Ros2vk	*Tetranychus urticae* Koch	99	99.57	0.00
8	Ros4mk	*Tetranychus urticae* Koch	100	99.49	0.00
9	Chr2mk	*Tetranychus urticae* Koch	100	98.61	0.00
10	Ger1vk	*Tetranychus urticae* Koch	100	99.32	0.00
11	Arpin1od	*Oligonychus biharensis* Koch	96	91.39	0.00
12	Ros3ap	*Oligonychus biharensis* Hirst	88	91.29	0.00

identified for all the species (Fig. 14, Table 7). Barcodes of different species at different alignment positions are furnished in and Table 8, 9, 10, 11, 12 and 13.

4.5.3. Pairwise distances of sequences

After aligning sequences of all the accessions using Clustal Omega, the pairwise distances were calculated using Kimura 2 Parameter model in MEGA X software. The result represents the variation in substitution levels between two sequences. The overall mean of the pairwise distance between all the sequences was 0.164. Intraspecific distance ranged from 0.00 to 0.036, while interspecific distance between species varied from 0.070 to 0.209.

With respect to interspecific distance, Ros1vk (*T. marianae*) showed maximum sequence divergence (0.194) from Ros3ap and Arpin1od (*O. biharensis*) and minimum (0.128) from Ros2vk (*T. urticae*). The accession Ros3ap (*O. biharensis*) showed maximum sequence divergence (0.204) from accession Ger1vk (*T. urticae*) and minimum divergence (0.135) from Ros2vk (*T. urticae*). The accession Arpin1od (*O. biharensis*) showed maximum divergence (0.201) from the accession Chr2mk (*T. urticae*) and minimum divergence (0.134) from Ros2vk (*T. urticae*). The accession Chr2mk (*T. urticae*) showed maximum divergence (0.209) from Zin1vk (*T. neocaledonicus*) and minimum divergence (0.098) from Mg1vk (*T. truncatus*). The accession Ger1vk (*T. urticae*) showed maximum divergence (0.217) from Zin1vk (*T. neocaledonicus*) and minimum divergence (0.105) from Mg1vk (*T. truncatus*). The accession Ros4mk (*T. urticae*) showed maximum divergence (0.202) from Zin1vk (*T. neocaledonicus*) and minimum divergence (0.089) from Cel1vk, Mg1vk and KR072563.1 (*T. truncatus*). The accession Ros2vk (*T. urticae*) showed maximum divergence (0.141) from AB736058 (*T. okinawanus*) and minimum (0.070) from Cel1vk and KR072563 (*T. truncatus*). The accession Zin1vk (*T. neocaledonicus*) showed maximum divergence (0.217) from Ger1vk (*T. urticae*) and minimum (0.121) from Ros2vk (*T. urticae*). The accession AB736059.1 (*T. okinawanus*) showed maximum divergence (0.169) from Ros3ap and Arpin1od (*O. biharensis*) and minimum (0.131) from Ros1vk (*Tetranychus* sp.1). The accession AB736058.1

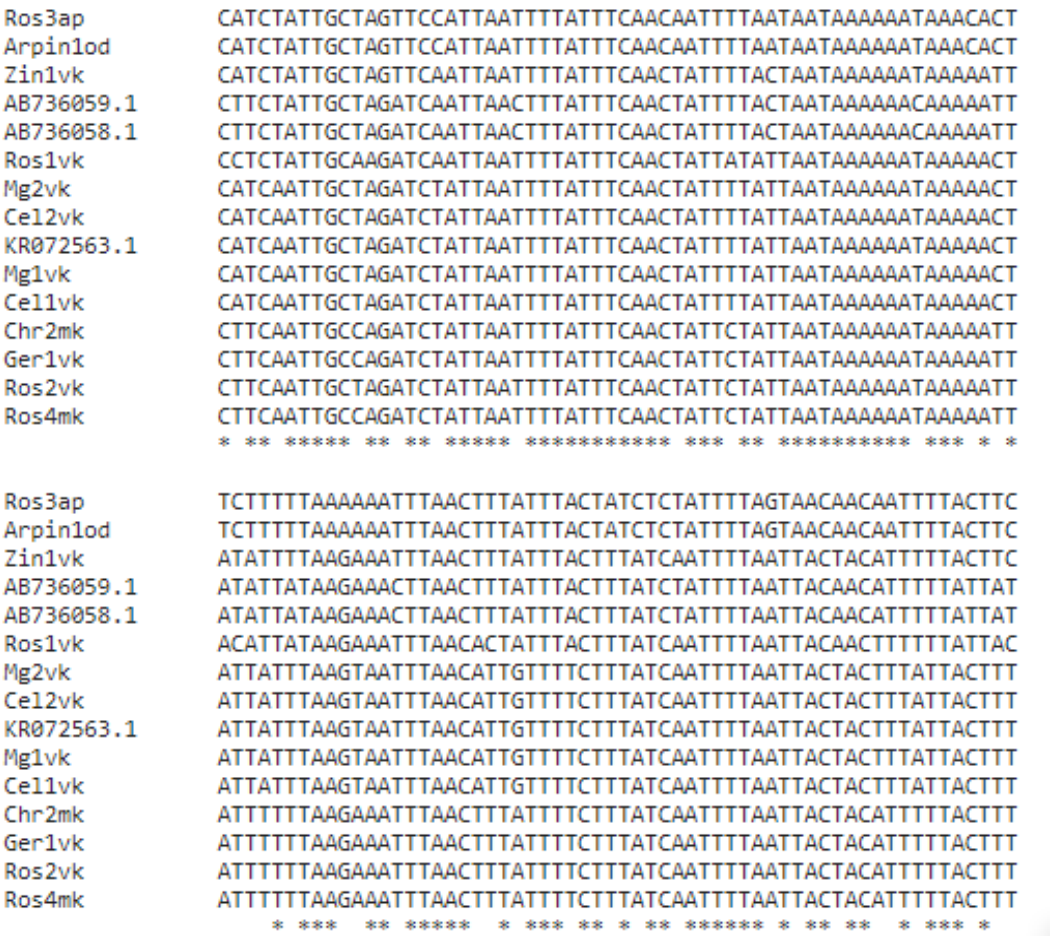


Fig. 14. Barcode gaps of *COI* sequences of spider mites

Table 7. Barcode gaps among the *COI* sequences of different accessions of spider mites

Mite species	Accession	Alignment positions																		
		173	180	183	198	207	219	222	225	240	252	264	286	290	293	299	300	302	303	310
Tetranychus urticae	Ros2vk	A	G	T	A	T	T	A	A	T	T	T	A	A	T	G	A	A	T	T
	Chr1vk	A	G	T	A	T	T	A	A	T	T	T	A	A	T	G	A	A	T	T
	Ger1vk	A	G	T	A	T	T	A	A	T	T	T	A	A	T	G	A	A	T	T
	Ros4vk	A	G	T	A	T	T	A	A	T	T	T	A	A	T	G	A	A	T	T
Tetranychus okinawanus	AB706359	A	A	T	A	C	T	T	A	T	C	T	A	A	T	G	A	A	C	T
	AB706358	A	A	T	A	C	T	T	A	T	C	T	A	A	T	G	A	A	C	T
Tetranychus neocaledonicus	Zin1vk	T	A	T	A	T	T	A	A	T	T	T	A	A	T	G	A	A	T	T
Tetranychus marianae	Ros1vk	A	A	T	G	T	T	A	T	A	T	T	A	A	T	G	A	A	T	C
Oligonychus biharensis	Ros3ap	A	A	G	A	T	A	A	A	T	T	A	C	T	T	A	A	G	T	T
	Arpin1od	A	A	G	A	T	A	A	A	T	T	A	C	T	T	A	A	G	T	T
Tetranychus truncatus	Mg1vk	A	A	T	A	T	T	A	A	T	T	T	A	A	A	G	T	A	T	T
	Cel2vk	A	A	T	A	T	T	A	A	T	T	T	A	A	A	G	T	A	T	T
	Mg2vk	A	A	T	A	T	T	A	A	T	T	T	A	A	A	G	T	A	T	T
	Cel1vk	A	A	T	A	T	T	A	A	T	T	T	A	A	A	G	T	A	T	T
	KR072563	A	A	T	A	T	T	A	A	T	T	T	A	A	A	G	T	A	T	T

…...Table 7. Barcode gaps among the *COI* sequences of different accessions of spider mites

Mite species	Accession	Alignment positions																	
		312	319	321	331	333	340	342	351	367	369	381	388	391	393	420	431	441	447
Tetranychus urticae	Ros2vk	A	T	A	A	T	T	T	A	T	A	T	A	T	A	T	G	A	A
	Chr1vk	A	T	A	A	T	T	T	A	T	A	T	A	T	A	T	G	A	A
	Ger1vk	A	T	A	A	T	T	T	A	T	A	T	A	T	A	T	G	A	A
	Ros4vk	A	T	A	A	T	T	T	A	T	A	T	A	T	A	T	G	A	A
Tetranychus okinawanus	AB706359	A	T	A	A	T	T	T	A	C	T	T	A	T	A	T	C	G	T
	AB706358	A	T	A	A	T	T	T	A	C	T	T	A	T	A	T	C	G	T
Tetranychus neocaledonicus	Zin1vk	A	T	A	A	T	T	T	A	T	A	T	G	T	A	T	G	A	A
Tetranychus marianae	Ros1vk	A	T	A	A	T	T	T	A	T	A	T	A	T	A	T	G	A	A
Oligonychus biharensis	Ros3ap	A	A	C	G	A	A	T	T	T	A	C	A	A	T	C	G	A	A
	Arpin1od	A	A	C	G	A	A	T	T	T	A	C	A	A	T	C	G	A	A
Tetranychus truncatus	Mg1vk	G	T	A	A	T	T	A	A	T	A	T	A	T	A	T	G	A	A
	Cel2vk	G	T	A	A	T	T	A	A	T	A	T	A	T	A	T	G	A	A
	Mg2vk	G	T	A	A	T	T	A	A	T	A	T	A	T	A	T	G	A	A
	Cel1vk	G	T	A	A	T	T	A	A	T	A	T	A	T	A	T	G	A	A
	KR072563	G	T	A	A	T	T	A	A	T	A	T	A	T	A	T	G	A	A

…...Table 7. Barcode gaps among the *COI* sequences of different accessions of spider mites

Mite species	Accession	Alignment positions																		
		451	453	463	474	486	489	499	504	516	522	531	535	537	546	547	549	567	574	577
Tetranychus urticae	Ros2vk	T	A	T	T	A	A	T	T	C	A	T	A	T	T	T	A	T	A	T
	Chr1vk	T	A	T	T	A	A	T	T	C	A	T	A	T	T	T	A	T	A	T
	Ger1vk	T	A	T	T	A	A	T	T	C	A	T	A	T	T	T	A	T	A	T
	Ros4vk	T	A	T	T	A	A	T	T	C	A	T	A	T	T	T	A	T	A	T
Tetranychus okinawanus	AB706359	C	T	T	T	T	A	T	T	T	A	T	A	T	T	T	A	T	A	T
	AB706358	C	T	T	T	T	A	T	T	T	A	T	A	T	T	T	A	T	A	T
Tetranychus neocaledonicus	Zin1vk	T	A	C	T	A	G	C	T	T	A	T	A	T	T	C	T	T	G	A
Tetranychus marianae	Ros1vk	T	A	T	T	A	A	T	C	T	A	T	A	T	C	T	A	C	A	T
Oligonychus biharensis	Ros3ap	T	A	T	C	A	A	T	T	T	A	T	T	A	T	T	A	T	A	T
	Arpin1od	T	A	T	C	A	A	T	T	T	A	T	T	A	T	T	A	T	A	T
Tetranychus truncatus	Mg1vk	T	A	T	T	A	A	T	T	T	G	C	A	T	T	T	A	T	A	T
	Cel2vk	T	A	T	T	A	A	T	T	T	G	C	A	T	T	T	A	T	A	T
	Mg2vk	T	A	T	T	A	A	T	T	T	G	C	A	T	T	T	A	T	A	T
	Cel1vk	T	A	T	T	A	A	T	T	T	G	C	A	T	T	T	A	T	A	T
	KR072563	T	A	T	T	A	A	T	T	T	G	C	A	T	T	T	A	T	A	T

…...Table 7. Barcode gaps among the *COI* sequences of different accessions of spider mites

Mite species	Accession	Alignment positions																	
		579	585	597	601	602	604	605	607	645	648	657	672	681	684	687	690	705	708
Tetranychus urticae	Ros2vk	T	G	A	A	T	G	G	T	T	A	T	T	A	T	T	T	C	T
	Chr1vk	T	G	A	A	T	G	G	T	T	A	T	T	A	T	T	T	C	T
	Ger1vk	T	G	A	A	T	G	G	T	T	A	T	T	A	T	T	T	C	T
	Ros4vk	T	G	A	A	T	G	G	T	T	A	T	T	A	T	T	T	C	T
Tetranychus okinawanus	AB706359	A	A	A	A	T	G	G	T	A	T	T	T	A	T	A	T	T	T
	AB706358	A	A	A	A	T	G	G	T	A	T	T	T	A	T	A	T	T	T
Tetranychus neocaledonicus	Zin1vk	A	A	A	T	C	A	T	T	A	A	T	T	A	T	T	T	T	T
Tetranychus marianae	Ros1vk	A	A	A	A	T	G	G	C	A	A	T	T	A	T	T	T	T	A
Oligonychus biharensis	Ros3ap	A	A	T	A	T	G	G	T	A	A	C	T	A	A	T	T	T	T
	Arpin1od	A	A	T	A	T	G	G	T	A	A	C	T	A	A	T	T	T	T
Tetranychus truncatus	Mg1vk	A	A	A	A	T	G	G	T	A	A	T	T	T	T	T	A	T	T
	Cel2vk	A	A	A	A	T	G	G	T	A	A	T	T	T	T	T	A	T	T
	Mg2vk	A	A	A	A	T	G	G	T	A	A	T	T	T	T	T	A	T	T
	Cel1vk	A	A	A	A	T	G	G	T	A	A	T	T	T	T	T	A	T	T
	KR072563	A	A	A	A	T	G	G	T	A	A	T	T	T	T	T	A	T	T

Table 8. Barcodes identified in *COI* sequence of the species *Tetranychus urticae*

Sl. No.	Alignment position	Nucleotide substitution
1	180	A→G
2	516	T→C
3	579	A→T
4	585	A→G
5	645	A→T
6	705	T→C

Table 9. Barcodes identified in *COI* sequence of the species *Tetranychus neocaledonicus*

Sl. No.	Alignment position	Nucleotide substitution
1	173	A→T
2	388	A→G
3	463	T→C
4	489	A→G
5	499	T→C
6	547	T→C
7	549	A→T
8	574	A→G
9	577	T→A
10	601	A→T
11	602	T→C
12	604	G→A
13	605	G→T

Table 10. Barcodes identified in *COI* sequence of the species *Tetranychus okinawanus*

Sl. No.	Alignment position	Nucleotide substitution
1	207	T⟶C
2	222	A⟶T
3	252	T⟶C
4	303	T⟶C
5	367	T⟶C
6	369	A⟶T
7	431	G⟶C
8	441	A⟶G
9	447	A⟶T
10	451	T⟶C
11	453	A⟶T
12	486	A⟶T
13	687	T⟶A

Table 11. Barcodes identified in *COI* sequence of the species *Tetranychus marianae*

Sl. No.	Alignment position	Nucleotide substitution
1	198	A⟶G
2	225	A⟶T
3	240	T⟶A
4	310	T⟶C
5	504	T⟶C
9	546	T⟶ C
7	567	T⟶C
8	708	T⟶A

Table 12. Barcodes identified in *COI* sequence of the species *Tetranychus truncatus*

Sl. No.	Alignment position	Nucleotide substitution
1	293	T⟶A
2	300	A⟶T
3	312	A⟶G
4	342	T⟶A
5	522	A⟶G
6	531	T⟶C
7	690	T⟶A

Table 13. Barcodes identified in *COI* sequence of the species *Oligonychus biharensis*

Sl. No.	Alignment position	Nucleotide substitution
1	183	T⟶G
2	219	T⟶A
3	264	T⟶A
4	286	A⟶C
5	290	A⟶T
6	299	G⟶A
7	302	A⟶G
8	319	T⟶A
9	321	A⟶C
10	331	A⟶G
11	333	T⟶A
12	340	T⟶A
13	351	A⟶T
14	381	T⟶C
15	391	T⟶A
16	393	A⟶T
17	420	T⟶C
18	474	T⟶C
19	535	A⟶T
20	537	T⟶A
21	597	A⟶T
22	684	T⟶A

(*T. okinawanus*) showed maximum divergence (0.176) from Ros3ap (*O. biharensis*) and minimum (0.137) from Ros1vk (*T. marianae*).

Genetic divergence was found to be in the range of 0.00 to 0.007 within *T. truncatus* and 0.003 to 0.023 within *T. urticae*. The species *T. okinawanus* and *O. biharensis* showed intraspecific nucleotide divergence of 0.004 and 0.036, respectively.

Interspecific divergence of *T. urticae* with *T. truncatus* ranged between 0.070 to 0.109, while the divergence with *T. okinawanus* ranged between 0.134 to 0.166. *Tetranychus urticae* showed an interspecific divergence ranging between 0.121 to 0.217 and 0.134 to 0.204 with the species *T. neocaledonicus* and *O. biharensis*, respectively. Interspecific divergence of *T. okinawanus* with all other species of spider mites collected in the study ranged between 0.131 to 0.176. Its divergence with *T. truncatus* ranged between 0.152 to 0.162 (Fig. 15).

4.5.4. Phylogeny analysis

Phylogenetic tree was constructed with MEGA X software using Maximum Likelihood method with bootstrap value of 500. Twelve *COI* sequences of spider mites generated in the study and four accessions of *T. truncatus* (KT208292, KR072563, KX669023 and KR271024), two accessions of *O. biharensis* (AB683678 and AB683679), one accession of *T. urticae* (AJ316605), one accession of *T. neocaledonicus* (AB736057), two accessions of *T. okinawanus* (AB736058 and AB736059) and one accession of *T. puerariсola* (KU516070) retrieved from NCBI database were used to construct the phylogenetic tree. Though *T. puerariсola* was not recorded in the study, as one accession (Ros1vk) showed 87.97 per cent similarity with *T. puerariсola* on BLASTn analysis in NCBI, a sequence of *T. puerariсola* retrieved from NCBI was used for phylogenetic analysis for the construction of tree. The phylogenetic tree formed two different clades. The sequences of four accessions of *T. truncatus* generated in the study, (Cel1vk, Cel2vk, Mg1vk and Mg2vk), four retrieved sequences of *T. truncatus* (KT208292, KR072563, KX669023, and KR271024),

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Ros1vk T. marianae															
2. Ros3ap O. biharensis	0.194														
3. Arpin1od O. biharensis	0.194	0.036													
4. Chr2mk T. urticae	0.168	0.200	0.200												
5. Ger1vk T. urticae	0.177	0.204	0.201	0.020											
6. Ros4mk T. urticae	0.158	0.191	0.191	0.015	0.013										
7. Ros2vk T. urticae	0.128	0.135	0.134	0.015	0.023	0.003									
8. Zin1vk T.neocaledonicus	0.179	0.203	0.185	0.209	0.217	0.202	0.121								
9. AB736059.1 T. okinawanus	0.131	0.169	0.169	0.156	0.159	0.148	0.134	0.149							
10. AB736058.1 T. okinawanus	0.137	0.176	0.175	0.162	0.166	0.155	0.141	0.155	0.004						
11. Cel2vk T. truncatus	0.133	0.149	0.152	0.102	0.109	0.091	0.074	0.158	0.153	0.160					
12. Cel1vk T. truncatus	0.142	0.168	0.168	0.100	0.107	0.089	0.070	0.173	0.156	0.162	0.004				
13. Mg1vk T. truncatus	0.134	0.148	0.150	0.098	0.105	0.089	0.071	0.160	0.153	0.160	0.002	0.000			
14. Mg2vk T. truncatus	0.143	0.150	0.156	0.106	0.113	0.097	0.071	0.166	0.153	0.160	0.007	0.006	0.005		
15. KR072563.1 T. truncatus	0.150	0.169	0.166	0.099	0.106	0.089	0.070	0.182	0.152	0.159	0.004	0.003	0.000	0.006	

Fig. 15. Pairwise distance of the *COI* sequences of spider mites

four accessions of *T. urticae* in the study (Ros2vk, Ger1vk, Chr2mk, Ros4mk), one retrieved sequence of *T. urticae* (AJ316605) and the retrieved sequence of *T. puerariсola* (KU516070) formed clade 1. Sequences of two accessions of *O. biharensis* in the study (Ros3ap, Arpin1od), two retrieved sequences of *O. biharensis* (AB683678 and AB683679), sequence of one accession of *T. neocaledonicus* in the study (Zin1vk), one retrieved sequence of *T. neocaledonicus* (AB736057), one sequence of *T. marianae* (Ros1vk) in the study, and two retrieved sequences of *T. okinawanus* (AB736058, AB736059) formed clade 2.

Clade 1 formed two subclades with all the accessions of *T. truncatus*, clustered on one branch, subclade 1, while all the accessions of *T. urticae* and one retrieved sequence of *T. puerariсola* formed subclade 2. However, in subclade 2 the accession, *T. puerariсola* formed an outgroup of *T. urticae* with a success rate 99.

In clade 2, two subclades were formed with a success rate 99. All the sequences of *O. biharensis* formed subclade 1 with a success rate 100, while the sequences of *T. neocaledonicus*, *T. okinawanus*, and *T. marianae* formed subclade 2 with a success rate 87. However, *T. marianae* formed an outgroup of *T. okinawanus* with success rate 86 (Fig. 16).

4.5.5. Submission to BOLD

The 12 sequences of five different species amplified with COI primer were submitted to Barcode of Life Data System (BOLD) to generate DNA barcodes. The process ID generated from BOLD for the 12 sequences are furnished in Table 14. The illustrative barcodes generated for 12 sequences representing five species in the study are furnished in Fig. 17.

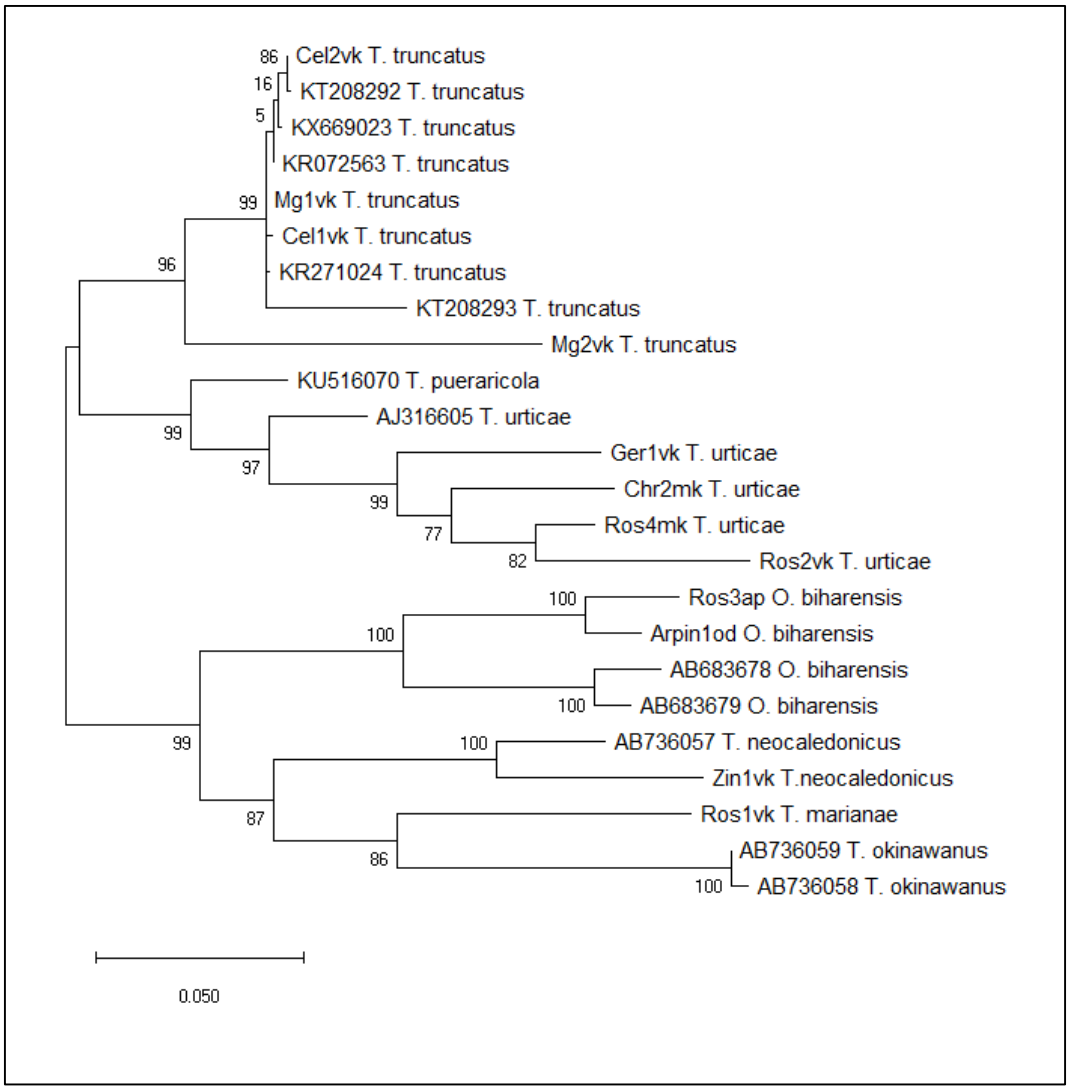
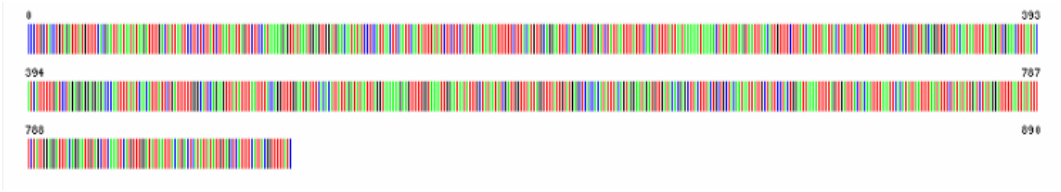


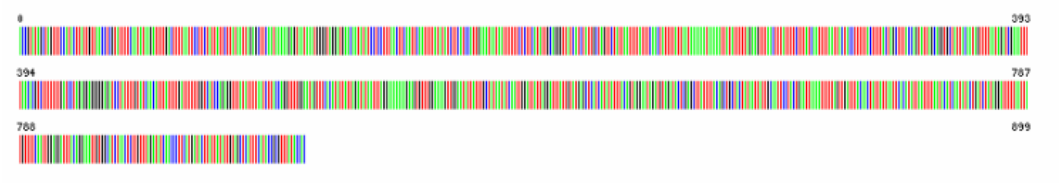
Fig. 16. Phylogenetic tree of *COI* sequences of spider mites

Table 14. Process IDs of the accessions submitted to BOLD

Sl. No.	Accession number	Process ID
1	Cel1vk	TETON001-19
2	Cel2vk	TETON002-19
3	Mg1vk	TETONOO3-19
4	Mg2vk	TETONOO4-19
5	Zin1vk	TETON005-19
6	Ros2vk	TETON006-19
7	Ger1vk	TETON007-19
8	Chr2mk	TETON008-19
9	Ros4mk	TETON009-19
10	Ros3ap	TETON010-19
11	Arpin1od	TETON011-19
12	Ros1vk	TETON012-19

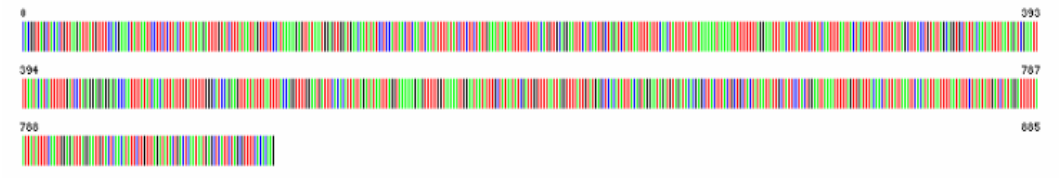


Cel1vk



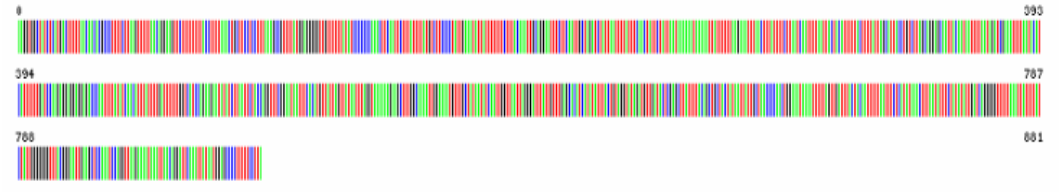
Cel2vk

Mg1vk



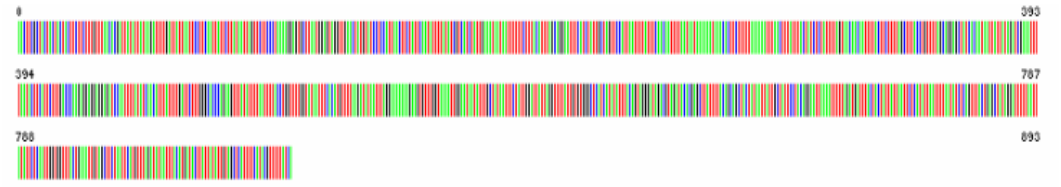
Mg2vk

Zin1vk

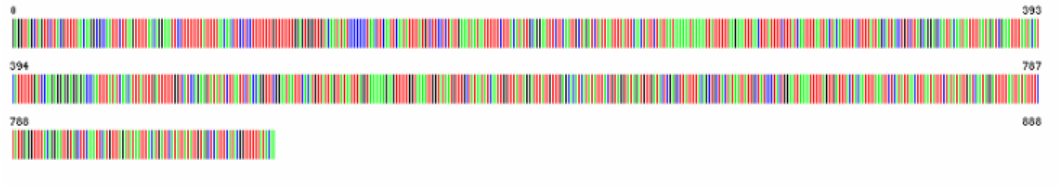


Ros2vk

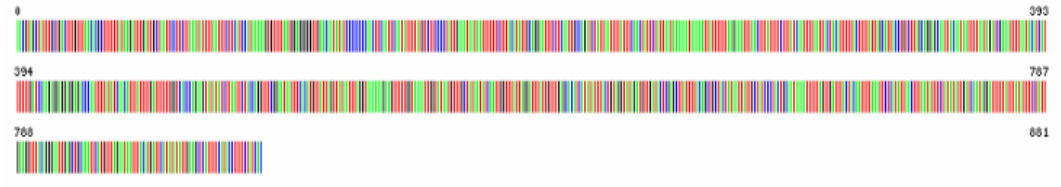
Fig. 17. Illustrative barcodes generated for the spider mite sequences



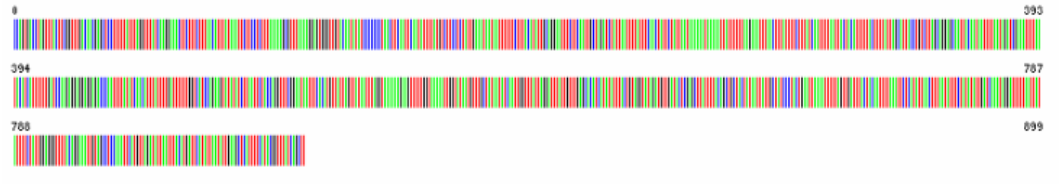
Ros3ap



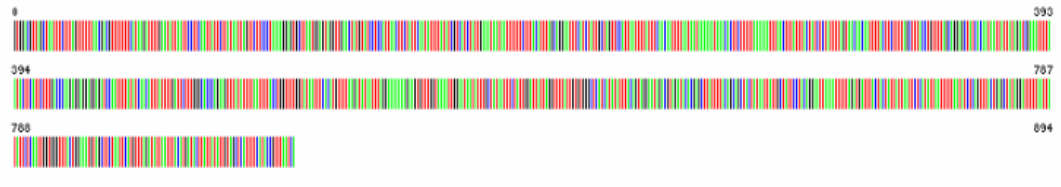
Ros4mk



Ger1vk

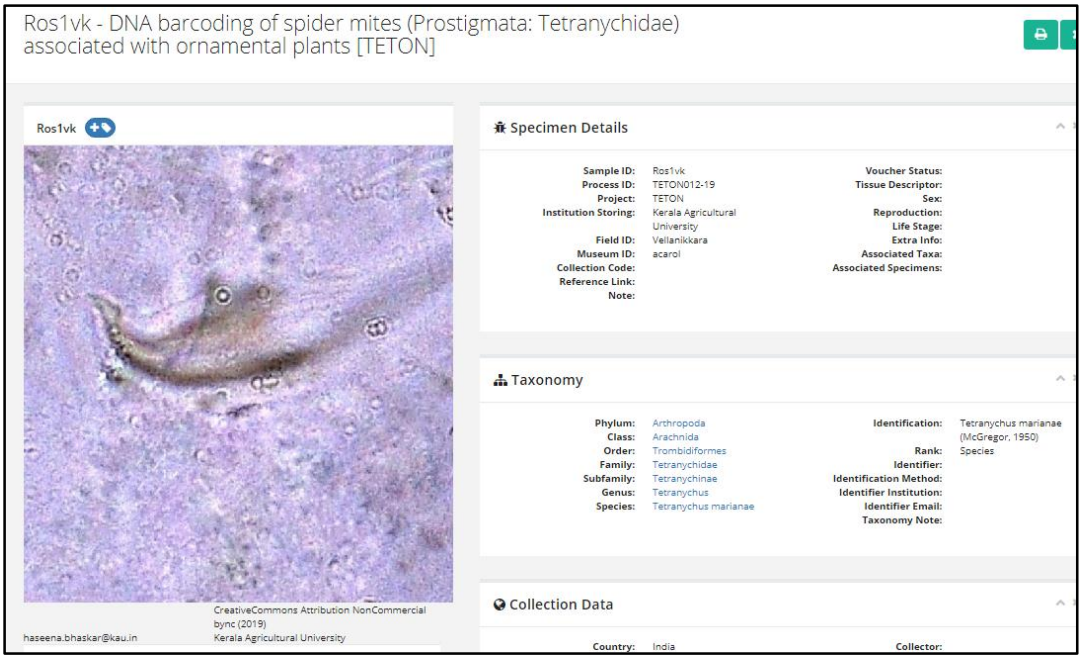


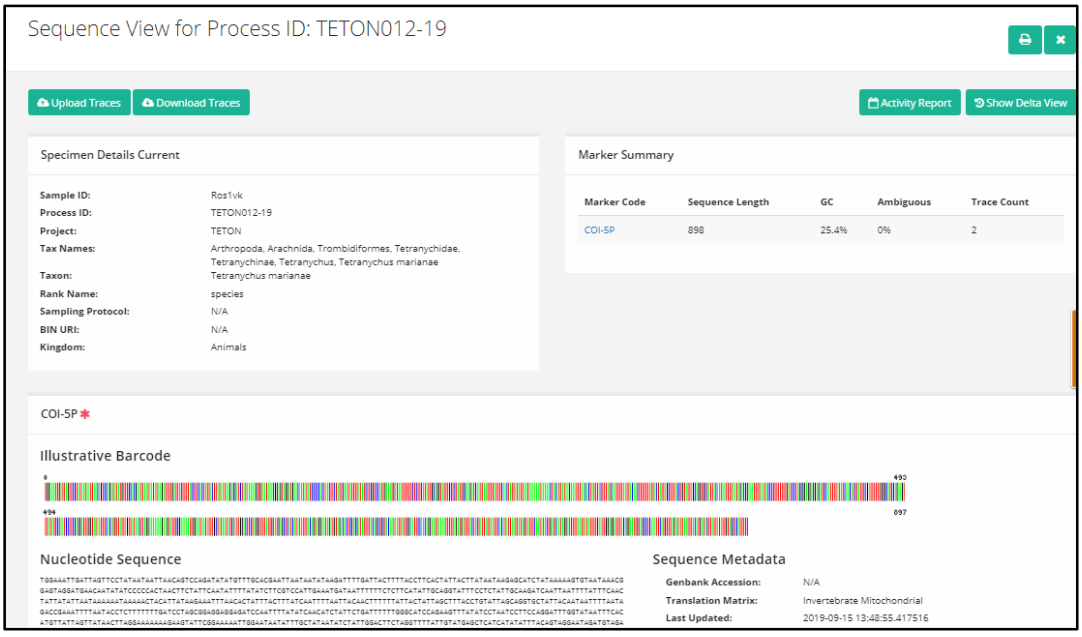
Chr2mk



Arpin1od

…..Fig. 17. Illustrative barcodes generated for the spider mite sequences





…..Fig. 17. Illuatrative barcode generated for the species *T. marianae*

Discussion

5. DISCUSSION

The results of the study entitled 'DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants' is discussed critically using the available literature.

Species identification of pests is the basis of understanding species diversity and also important to develop pest control strategies.

5.1. Diversity of spider mites on ornamental plants

The study documented six species of spider mites under two genera associated with 12 ornamental plants from Thrissur and Ernakulam districts. Five species of *Tetranychus* Dufour *viz.*, *T. okinawanus* Ehara, *T. truncatus* Ehara, *T. urticae* Koch, *T. neocaledonicus* Andre and *T. marianae* McGregor and one species of *Oligonychus* Berlese *viz*., *O. biharensis* Hirst were recorded in the study. In the first part of the study, spider mites collected on different host plants from different locations were identified examining the morphological features. The two genera *Tetranychus* and *Oligonychus* documented in the study could be distinguished based on the structure of empodium and position of duplex setae on tarsus I. Gupta (1985) in his book 'Plant Mites of India' provided key for the identification of different genera of Tetranychidae, in which morphology of empodium and duplex setae were used to differentiate *Oligonychus* from *Tetranychus* and other tetranychid genera.

The species of *Tetranychus* were identified based on the morphology of aedeagus, particularly the structure of aedeagal knob. Gupta (1985) and Gupta and Gupta (1994) in their description of species of Tetranychidae provided the detailed structure of aedeagus of different species with diagrams. This formed the reference material to identify the species *T. urticae*, *T. neocaledonicus* and *O. biharensis*. *Tetranychus truncatus* was identified based on the key taxonomic characters furnished by Srinivasa *et al*. (2012), while *T. okinawanus* was identified based on the description of Ehara (1995) and *T. marianae* based on the description of Zeity *et al.* (2016).

The study recorded *T. okinawanus* as the predominant mite species on ornamental plants. It was recorded on balsam, chrysanthemum, rose, *Adenium*, *Gerbera*, marigold, orchid and cairo morning glory. Ehara (1995) reported the species for the first time on *Pueraria lobata* from Okinawa Islands of Japan. Later it was known from Japan and Taiwan on more than 90 host plants. This species was reported for the first time from India on the ornamental plant, *Adenium obesum* from a nursery in Thrissur district in Kerala (Zeity *et al.*, 2016). Later it was reported on cucumber (Bennur *et al.*, 2015; Lenin *et al.*, 2015 and Lenin and Bhaskar, 2016). Arunima *et al.* (2017) reported four new host plants for this species *viz*., papaya, ashgourd, brinjal and cowpea from India. The present study reports seven new hosts for *T. okinawanus* from India *viz*., rose, *Gerbera*, marigold, cairo morning glory, balsam, orchid and chrysanthemum. This is an indication of invasive nature of *T. okinawanus*.

In the study, *T. truncatus* was recorded on ornamental plants marigold, cock's comb, and rose from six different localities of Thrissur district. These are new host records for *T. truncatus* from India. In India, *T. truncatus* was first reported from Northwestern Himalayan regions of Jammu and Kashmir and Himachal Pradesh (Rather, 1983). Long years later, it was reported from Karnataka by Srinivasa *et al.* (2012) on mulberry. Bennur *et al*. (2015) reported this as a predominant species of spider mite on many vegetable crops in Kerala. The mite also infests cucumber and amaranthus grown in polyhouses (Lenin and Bhaskar, 2016). Later, Arunima (2017) reported *T. truncatus* on cowpea, pumpkin, tapioca, banana and *Dahlia* from different regions of Kerala. Bhaskar and Lenin (2018) reported *T. truncatus* as a serious pest of banana (Nendran) in Kerala. It was also reported to infest brinjal in Kerala (Lekha and Kinathi, 2019). The species *T. truncatus* is a polyphagous pest and 90 plant species in 32 families have been reported from world-over as host plants (Spider Mite Web, 2019). The three host plants of *T. truncatus* recorded in the current study are new host records from India.

The present study recorded *T. urticae* Koch on rose, chrysanthemum and *Gerbera* from different localities of Thrissur district. The two spotted spider mite, *T. urticae* was first described by Koch in 1836 (Pritchard and Baker, 1955), and later found to be distributed throughout the tropical and sub tropical parts of the world (Jeppson *et al*., 1975). Outbreaks of *T. urticae* infestation on lady's finger and beans in Bangladesh has been reported by Gapud (1981). Biswas *et al.* (2004) reported that the mite infests vegetable crops and ornamental plants in Bangladesh. Tehri (2014) documented pest status of *T. urticae* on greenhouse vegetables, ornamental and horticultural crops worldwide and reported its polyphagous nature. The spider mite, *T. urticae* is a serious pest on rose grown in polyhouse and open condition in Navsari, Gujarat (Desai *et al*., 2017). In Kerala, the two spotted spider mite, *T. urticae* was reported as a predominant species on vegetable crops *viz*., brinjal, bhindi, amaranthus and cowpea (Sudharma and Nair, 1999; Binisha and Bhaskar, 2013). Lekha and Kinathi (2019) reported *T. urticae* on brinjal, moringa and winged bean from Northern districts of Kerala. However, recent studies by All India Network Project on Agricultural Acarology to document spider mite diversity on crops of Kerala during 2013- 2018 did not record *T. urticae* on vegetable crops. But in this study, *T. urticae* was found infesting rose grown both under polyhouse as well as on *Gerbera* and chrysanthemum under open condition.

The mite species, *Tetranychus neocaledonicus* Andre was recorded on ornamental plant *Zinnia* from Vellanikkara, Thrissur. It is a cosmopolitan species in tropical and subtropical areas, infesting a wide variety of agricultural plants (Pritchard and Baker 1955: Bolland *et al.,* 1998). It was reported in India by Khot and Patel (1956), later by Manson (1963), Nassar and Ghai (1981) Gupta (1992); Gupta and Gupta (1994); Gupta (1995); Gupta and Chatterjee (1997) and Migeon (2015). Recently, Lekha and Kinathi (2019) reported *T. neocaledonicus* on brinjal, tomato and okra from Kerala. The reported host range of *T. neocaledonicus* include *Chrysanthemum* sp., *Dahlia* sp., *Gerbera* sp., *Helianthus annuus, Tagetes erecta, Gladiolus* sp., *Bauhinia* sp., *Bougainvillea* sp., *Jasminum* sp. and *Arachis pintoi* (Spider Mite Web, 2019).

The study recorded the mite species, *T. marianae* on rose from Vellanikkara. This is the first record of the species from Kerala. *Tetranychus marianae* was first described by McGregor in 1950 from USA. Later it was reported from 71 different host plants from different countries (Bolland *et al.*, 1998). In India it was first reported from Karnataka recently by Zeity *et al*. (2016) on *Centrocema pubescence*, and later only from this study.

In the study, *Oligonychus biharensis* was recorded on rose, *Bauhinia* and pinto peanut. The mite is a native of India and described by Hirst in 1924. It was later reported by Nassar and Ghai (1981); Gupta (1992); Gupta and Gupta (1994). Its host range among ornamental plants include rose, *Bauhinia* and hibiscus (Spider Mite Web, 2019).

5.2. Molecular characterisation

The present study was conducted to generate barcodes of spider mite species infesting major ornamental plants. In the study, mitochondrial *COI* gene is used for species delineation.

5.2.1. Isolation of DNA and PCR amplification

In the present study, DNA was isolated from spider mite accessions following CTAB method (Rogers and Benedich, 1994) with some modifications. CTAB method was suggested as an effective method for DNA extraction of mites (Desloire *et al*., 2006). Adult female mites (10-15) were used for the isolation. The crushed samples were kept for 1 h for incubation at 60 °C, instead of 25 minutes suggested by Rogers and Benedich (1994). For DNA precipitation, on adding ethanol and sodium acetate buffer, the samples were incubated overnight instead of 15-20 minutes suggested by Rogers and Benedich (1994). As suggested by Bennur (2015) the DNA precipitate was washed two times using 70 and 100 per cent ethanol. NanoDrop spectrophotometer showed that the absorbance ratio of the isolated DNA from different samples ranged from 1.93 to 2.14. The absorbance value above 2 indicates RNA contamination. In this study, absorbance ratio for ten out of 12 DNA samples showed absorbance value above 2. However,

COI loci of all the samples could be successfully amplified by PCR and the fragment length ranged between 800 to 900 bp indicating the good quality of isolated DNA. Ahaniazad *et al*. (2018) isolated DNA of oribatid mite following CTAB method and found that absorbance ratio ranged from 1.9 - 2.33. However, he could successfully amplify 350 bp fragment of the nuclear gene 28S rDNA in samples showing absorbance ratio above 2 and reported the good quality of DNA.

In the present study *COI* loci were successfully amplified using the protocol of Li *et al*. (2010), for species delineation of mite accessions. Evolutionary studies on mites have mainly surveyed the mitochondrial *Cytochrome Oxidase subunit I* gene (*COI*) (Navajas and Fenton, 2000). The two main genomic DNA regions targeted to identify *Tetranychus* species from other taxa of spider mite species are the *Internal Transcribed Spacer 2* (*ITS2*) rRNA gene region of the long fragments of 714-817 bp and *Cytochrome Oxidase I* (*CO*I) of the mitochondria of 900bp (Matsuda *et al.*, 2013). Bennur *et al*. (2015) also reported that *ITS*2 and *COI* are effective markers in DNA barcoding of tetranychid spider mites.

5.2.2. Sequence analysis using *in- silico* tools

Nucleotide BLAST (BLASTn) is a programme that compares nucleotide query sequence to a database of nucleotide sequences. It is a heuristic that finds short matches between two sequences, attempts to start alignments from these 'hot spots' and provides statistical information about an alignment (Johnson *et al*., 2008)

The mitochondrial *COI* sequences of spider mite accessions, generated in the study were compared with the existing sequences in the NCBI database using BLASTn programme. Homology search of 12 sequences in BLASTn identified four species of spider mites *viz*., *T. truncatus, T. urticae, T. neocaledonicus* and *Oligonychus biharensis*. The accessions Cel1vk, Cel2vk, Mg1vk and Mg2vk showed maximum similarity, with the sequences of *T. truncatus*. Cel1vk and Cel2vk showed maximum similarity and query coverage with the accession

KR072563 and KR052245 representing *T. truncatus* on amaranthus from Thrissur district, Kerala reported by Bennur *et al*. (2015). Mg1vk showed maximum similarity per cent and query coverage with the accession MF774629, while Mg2vk showed maximum similarity per cent with accession MF774634 reported as *T. truncatus* by Arunima *et al*. (2017) from Kerala. Morphological characterisation in this study also revealed the species identity of these accessions as *T. truncatus*.

The accessions Ros2vk, Ger1vk, Chr2mk and Ros4mk showed high similarity per cent with the accession, MK508722, *Tetranychus urticae* reported by Inak *et al*. (2019). These accessions were identified as *T. urticae*, based on morphology in the study.

The accession Zin1vk showed maximum similarity with the accession AB736057 representing the species, *T. neocaledonicus*, reported by Matsuda *et al*. (2013) from Japan confirming the morphological identification in the study.

The accessions Ros3ap and the accession Arpin1od showed maximum sequence similarity with the accession AB683679, representing *O. biharensis* reported by Matsuda *et al*. (2012) from Japan. Morphology based identification also established the species identity of these accessions as *O. biharensis*.

The accession, Ros1vk identified as *T. marianae* based on morphological characterization did not show any similarity with sequences in the NCBI database. In BLAST analysis, the sequence did not show significant similarity with any of the sequences in the NCBI database. This shows that the *COI* sequence of *T. marianae* is not available in NCBI database. In the study, *COI* sequence of *T. marianae* was submitted for the first time in NCBI database

5.2.3. Barcode gap among different species

Interspecfic and intergeneric variations were examined among the species of spider mites in the study. The *COI* sequences of different species of spider

mites were analysed for variation in nucleotide composition to find out presence of barcode gap. The variation in the nucleotides that occur in all the members of a particular species, for any candidate locus is considered as barcode. These definite sequence variation is used for species discrimination. The locus, *COI* provides an attractive genetic barcode for species identification because of its high copy number, rapid rate of mutation, and ease of amplification or sequencing and alignment for intra- and interspecific comparisons (Smith *et al*., 2006). Variations between the species were also noted as transitions and transversions. On analysis of *COI* sequences, five species of spider mites showed that both transition and transversion substitutions occurred in the sequence. However, Navajas *et al*. (1996) who analysed *COI* sequences of 20 species of phytophagous mites found that most variations in sequences were transition substitutions, but transversions were also found. Arunima *et al*. (2017), in her study on barcode gap analysis of three species of spider mites found both transition and transversion substitutions in *COI* loci of spider mite species *T. udaipurensis* and *T. truncatus*. In the present study, approximately 150 barcode gaps were identified in *COI* sequence and it reveals the efficiency of COI markers for species discrimination of spider mites, particularly under genera, *Tetranychus* and *Oligonychus*.

5.2.4. Pairwise distance among different species of spider mites

In the present study, the range of pairwise distance was 0.000 to 0.217. Minimum intraspecific divergence (0.000) was shown between two accessions of *T. truncatus*, followed by *T. okinawanus* (0.004). Maximum intraspecific divergence (0.036) was seen between two accessions of *O. biharensis*.

The species of spider mite, *T. marianae* showed divergence value of 0.128 to 0.177 with *T. urticae*. It showed minimum divergence with *T. okinawanus*. In BLAST analysis, *T. marianae*, showed only similarity of 87. 97 per cent with *T. puearicola*. But in the pairwise distance analysis it showed maximum similarity with *T. okinawanus* and maximum divergence with *T. urticae*. However morphological characterisation in the study showed that *T. marianae* differ considerably from *T. okinawanus, T. urticae* and *T. puearicola*.

Tetranychus neocaledonicus showed maximum interspecific divergence with *T. urticae* and minimum with *T. okinawanus*. *Oligonychus biharensis* showed maximum interspecific divergence with both *T. truncatus* and *T. urticae*.

The genetic divergence among four accessions of *T. urticae* collected in the study ranged from 0.003 to 0.023.

Mutisya *et al*. (2018) studied the diversity of *COI* sequences of *T. urticae* infesting *Brachiaria* grass in Kenya and showed that there is no much intraspecific difference on *COI* region from the local populations of Kenya, while it showed genetic divergence of 0.111, 0.182 and 0.184 with *T. urticae* of China, Spain and France. All the four accessions of *T. urticae* in the study were collected from two neighbouring localities, Madakkathara and Vellanikkara in Thrissur district, representing local population of this region. This may be the reason why no much intraspecific variation is recorded in the *COI* region among the accessions.

5.2.5. Phylogenetic analysis

For phylogenetic analysis of DNA sequences, trees can be constructed by neighbor joining method as well as maximum likelihood method. In the present study, phylogenetic tree was constructed for 23 *COI* sequences of spider mites representing seven species using maximum likelihood method, with a maximum bootstrap value of 500. The tree formed two clades with all sequences of *T. truncatus*, *T. urticae* and one sequence of *T. puerariсola* grouping into a single clade. In the other clade, the sequences of *O. biharensis*, *T. neocaledonicus*, *T. okinawanus* and *T. marianae* were grouped.

In clade 1, *T. urticae* and *T. truncatus* formed two different subclades indicating the genetic divergence between the two species. Phylogenetic studies by Ros and Breeuwer (2007) on species of spider mites collected from six locations in Europe had revealed the genetic divergence between *T. truncatus* and *T. urticae* which supports the present study. In subclade 1, *T. puerariсola* and *T. urticae* clustered in one group indicating common ancestory (monophyly) of the

two species. Ehara and Gotoh (1996) had described the similarity of *T. urticae* and *T. puerariсola* morphologically. Monophyly of the two closely related species *viz*., *Panonychus citri* McGregor and *Panonychus osmanthi* Ehara and Gotoh, which morphologically and molecularly resembled each other was brought out in the phylogenetic analysis based on mitochondrial *COI* gene by constructing phylogenetic tree using maximum likelihood method.

In clade 2, the sequences of *O. biharensis* is clustered in a separate branch, while *T. neocaledonicus* and *T. marianae* clustered along with *T. okinawanus* in another branch. The *COI* sequences of *T. marianae* did not match significantly with any *COI* sequences of spider mites in the NCBI database. In BLAST analysis it had shown similarity with *T. puerariсola* only upto 87.97 per cent. However, in the phylogenetic tree constructed, the sequences of *T. puerariсola* and *T. marianae* were distributed in two different clades indicating the divergence of the two species. It shows that mitochondrial *cytochrome oxidase subunit I* (*COI*) locus of DNA in the species, *T. marianae* has not been characterised, so far, sequenced and deposited in the NCBI database. Phylogenetic analysis show monophyly of *T. okinawanus* and *T. marianae* supported with high bootstrap value of 86.

However, for a better understanding of phylogenetic relationship among different species of the family Tetranychidae, it is necessary to include DNA sequences of more species for analysis. In this study, the phylogenetic analysis was carried out only based on *COI* gene. Though the tree revealed several well supported clades, there is a need to examine nuclear genes also to understand the relationship among the different species.

5.2.6. Submission of sequences in BOLD

In this study, 12 *COI* sequences representing five species *viz*., *T. truncatus, T. urticae, T. neocaledonicus*, *T. marianae* and *O. biharensis*, were submitted in BOLD system and illustrative barcodes generated. Barcodes of the four species except *T. marianae* have been published earlier in BOLD system

based on sequences submitted from different countries including India. Maximum published records for spider mites in BOLD system is for *T. urticae* with 222 records from 18 countries. There are 75 published records for *T. truncatus* based on sequences submitted from three countries. For *T. neocaledonicus*, six barcodes are published in BOLD system from three countries *viz*., India, Australia and Japan, while only three barcodes are published for *O. biharensis*.

Summary

6. SUMMARY

The study entitled 'DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants' was carried out at the Department of Plant Biotechnology and All India Network Project on Agricultural Acarology, College of Horticullture during the period 2017-2019. The objectives of the study were to generate DNA barcodes for different species of spider mites infesting commercial ornamental plants of central Kerala and to find out the genetic variability among them. Survey was conducted in Thrissur and Ernakulam districts and spider mites associated with ornamental plants *viz*, rose, cock's comb, marigold, chrysanthemum, balsam, *Adenium*, *Gerbera*, *Bauhinia*, pinto peanut, cairo morning glory, orchid and *Zinnia* were collected. Isoline cultures of spider mites from different ornamental plants surveyed were maintained in the laboratory with unique accession numbers, for morphological and molecular characterization.

Salient findings of the study are furnished below:

1. Morphological characterization of mite specimens from different accessions revealed the presence of six species belonging to two genera *viz*., *Tetranychus* Dufour and *Oligonychus* Berlese
2. The genus *Tetranychus* was found to be more diverse with five species *viz*., *Tetranychus truncatus* Ehara, *Tetranychus okinawanus* Ehara, *Tetranychus urticae* Koch, *Tetranychus neocaledonicus* Andre and *Tetranychus marianae* McGregor
3. The genus *Oligonychus* was represented by only one species, *Oligonychus biharensis* Hirst
4. The mite species, *T. okinawanus* recorded wider host range with new host records from India *viz*., rose, chrysanthemum, *Gerbera*, marigold, cairo morning glory, balsam and orchid
5. The study recorded three new host plants for *T. truncatus viz*., rose, marigold and cock's comb

6. The mite species, *T. marianae* was recorded for the first time from Kerala
7. Sufficient quantity of DNA could be isolated from spider mite specimens when used at 10-15 numbers. Crushed samples on incubation at 60 °C for 1h followed by washing in 70 per cent and 100 per cent ethanol yielded good quality DNA
8. PCR amplification of *COI* region of spider mite DNA using universal primer yielded amplicons in the range of 800-900 bp, suitable for barcoding
9. Homology of the sequences of the spider mite accessions using BLASTn analysis showed 90 to 99 per cent similarity with sequences in NCBI database
10. The *COI* sequence of *T. marianae* did not show significant similarity with any of the accessions in NCBI database. Sequence of *T. marianae* was deposited in NCBI for the first time
11. Pairwise distance analysis of *COI* sequences showed intraspecific nucleotide divergence in the range 0.000 to 0.007 within *T. truncatus* and 0.003 to 0.023 within *T. urticae*. The species *T. okinawanus* and *O. biharensis* showed intraspecific nucleotide divergence of 0.004 and 0.036, respectively
12. Analysis of *COI* sequences of five species of spider mites showed that both transition and transversion occurred in the sequences confirming existence of barcode gaps in the locus to differentiate *T. urticae, T. okinawanus, T. neocaledonicus, O. biharensis* and *T. marianae* from *T. truncatus*
13. Phylogenetic tree constructed using 23 *COI* sequences of spider mites representing seven species formed two clades with sequences of *T. truncatus, T. urticae, T. puearicola* and *O. biharensis* grouping into one clade, while *T. neocaledonicus, T. okinawanus* and *T. marianae* in the other clade. Phylogenetic tree revealed monophyly of the species, *T. urticae* and *T. puearicola*

14. Phylogenetic analysis of the *COI* sequences also showed that the species, *T. marianae*, which has not shown similarity with any of the sequences in NCBI database is closely related to *T. okinawanus*
15. *COI* sequences of 12 accessions representing five species were submitted to BOLD System for generation of species barcodes. Sequence of *T. marianae* was submitted for the first time in BOLD System.

The study on diversity of spider mites based on morphological and molecular characterization revealed that *T. okinawanus* is the predominant species infesting ornamental plants in Kerala. The study has shown that rose harbours many species of spider mites, indicating the need for imposing strict quarantine regulations for movement of planting materials of rose to avoid entry and invasion of mites into new areas. The potential of *T. okinawanus* and *T. truncatus* to turn invasive in Kerala's ecosystems is also brought out. The study establishes the reliability of *COI* locus as a marker for species discrimination in spider mites.

Reference

REFERENCES

Ahaniazad, M., Bagheri, M., Roumi, V., and Akrami, M. A. 2018. An efficient and non-destructive DNA extraction method for oribatid mites. *Arch. Phytopathology Plant Protect.* 51(3): 187-196.

Anderson, D. L. and Trueman, J. W. H. 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Exp. Appl. Acarol*. 24: 165–189.

Arabuli, T. and Gotoh, T. 2018. A new species of spider mite, *Oligonychus neocastaneae* sp. nov. (Acari: Tetranychidae), from Japan. *Zootaxa* 4378(4): 563-572.

Arunima, V. 2017. DNA barcoding of spider mites (Prostigmata: Tetranychidae) on major crop plants of Kerala. M.Sc.(Ag) thesis, Kerala Agricultural University, Thrissur, 59p.

Ball, S. L., Hebert, P. D., Burian, S. K., and Webb, J. M. 2005. Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *J. N. Am. Benthol. Soc.* 24(3): 508-524.

Barrett, R. D. H. and Hebert, P. D. N. 2005. Identifying spiders through DNA barcodes. *Can. J. Zool*. 83: 481–491.

Bennur, S., Abida, P. S., Valsala, P. A., Mathew, D., and Bhaskar, H. 2015. DNA barcoding of spider mites (Prostigmata: Tetranychidae) in vegetables using *COI* and *ITS*2 markers. *Genome*, 58(5): 195.

Bennur, S. M., Abida, P. S., Haseena, B., Deepu, M., and Valsala, P. A. (2015): National Library of Medicine (US), National Centre for Biotechnology Information [1988]-Accession No. KR072563.1, *Tetranychus truncatus cytochrome oxidase subunit I* gene, partial cds, mitochondrial [cited 2019 June 25]. Available from: https://www.ncbi.nlm.nih.gov/nuccore/KR072563.1.

Bennur, S. M., Abida, P. S., Haseena, B., Deepu, M., and Valsala, P. A. (2015): National Library of Medicine (US), National Centre for Biotechnology Information [1988]- Accession No. KR052245, *Tetranychus truncatus*, , *cytochrome oxidase subunit I* gene, partial cds, mitochondrial [cited 2019 June 25]. Available from: https://www.ncbi.nlm.nih.gov/nuccore/KR052245.

Besansky, N. J., Severson, D. W., and Ferdig, M. T. 2003. DNA barcoding of parasites and invertebrate disease vectors: what you don't know can hurt you. *Trends Parasitol.* 19(12): 545-546.

Bhaskar, H. and Lenin, N. 2018. Management of banana leaf mite, *Tetranychus truncatus* (Prostigmata: Tetranychidae), a new pest of banana. Book of abstracts, National Seminar on Frontiers in Biological Research. March 12-14, 2018. Department of Zoology, University of Calicut, Kerala, 9p

Binisha, K. V. and Bhaskar, H. 2013. Mite fauna associated with major vegetable crops of Kerala. *Entomon*, 38(1): 47-52.

Bhaskar, H., Mallik, B., and Srinivasa, N. 2013. Report of orchid mite, *Tenuipalpus pacificus* Baker (Prostigmata: Tenuipalpidae) from Kerala, India. *Entomon*, 38(2): 111-114.

Biswas, G. C., Islam, W., Haque, M. M., Saha, R. K., Hoque, K. M. P., Islam, M. S., and Haque, M. E. 2004. Some biological aspects of carmine mite, *Tetranychus cinnabarinus* Boisd. (Acari: Tetranychidae) infesting egg-plant from Rajshahi. *J. biosci.* 4(5): 588-591.

Blouin, M. S., Yowell, C. A., Courtney, C. H., and Dame, J. B. 1998. Substitution bias, rapid saturation, and the use of mtDNA for nematode systematics. *Mol. Biol. Evol.* 15(12): 1719-27.

Bolland, H. R., Gutierrez, J., and Flechtmann, C. H. W. 1998. *A World Catalogue of the Spider Mite Family (Acari: Tetranychidae)*. Brill Academic Publishers, Netherlands, 392 p.

Caesar, R. M., Sörensson, M., and Cognato, A. I. 2006. Integrating DNA data and traditional taxonomy to streamline biodiversity assessment: an example from edaphic beetles in the Klamath ecoregion, California, USA. *Divers. Distrib.* 12(5): 483-489.

Cherian, M. C. 1931. South Indian Acarina. *J. Asiatic Soc.* 27: 141-147.

Childers, C. C. and Rodrigues, J. C. V. 2005. Potential pest mite species collected on ornamental plants from Central America at port of entry to the United States. *Fla Entomol*. *88*(4): 408-415.

Cullen, E. and Schramm, S. 2009. Two spotted spider mite management in soybean and corn. [on-line]. Available: http:// corn.agronomy.wisc.edu/ [13 July 2019].

Cywinska, A., Hunter, F. F., and Hebert, P. D. N. 2006. Identifying Canadian mosquito species through DNA barcodes. *Med. Vet. Entomol.* 20(4): 413-424.

Desai, S. R., Patel, K. G., and Shukla, A. 2017. Seasonal incidence of spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae) infesting rose under poly house condition. *Int. J. Curr. Microbiol. App. Sci*. 6(9): 2661-2669.

Desloire, S., Moro, C. V., Chauve, C., and Zenner, L. 2006. Comparison of four methods of extracting DNA from *D. gallinae* (Acari: Dermanyssidae). *Veterinary Research*, 37(5): 725-732.

Dhooria, M. S. 1999a. Two spotted spider mite (*Tetranychus urticae*) - a serious pest of roses in polyhouses and its control. *J. Acarol*. 14(1&2): 84-87.

Dhooria, M. S. 1999b. Observation on the status of phytophagous and predacious mites on different ornamental plants in Punjab. *J. Acarol*. 14(1&2): 88-89.

Dhooria, M. S. 2016. *Fundamentals of Applied Acarology*. Springer Publishers, New York, 470p.

Din, N. and Engberg, J. 1979. Extrachromosomal ribosomal RNA genes in *Tetrahymena* structure and evolution. *J. Mol. Biol.* 134: 555-574.

Dona, J., Real, J. D., Mironov, S., Bazaga, P., Serrano, D., and Jovani, R. 2015. DNA barcoding and minibarcoding as a powerful tool for feather mite studies. *Mol. Ecol. Resour.* 15(5): 1216-1225.

Dove, C. J., Rotzel, N. C., Heacker, M., and Weigt, L. A. 2008. Using DNA barcodes to identify bird species involved in birdstrikes. *J. Wildl. Manag.* 72(5): 1231-1236.

Eggert, L. S., Rasner, C. A., and Woodruff, D. S. 2002. The evolution and phylogeography of the African elephant inferred from mitochondrial DNA sequence and nuclear microsatellite markers. *Proc. Roy. Soc. Biol. Sci.* 269: 1993–2006.

Ehara, S. (1995) A New Species of *Tetranychus* (Acari, Tetranychidae) from the Ryukyu Islands. *Jpn J. Entomol*. 63 (1): 229-233.

Ehara, S. and Gotoh, T. 1996. Two new species of spider mites occurring in Japan (Acari, Tetranychidae). *J. Acarol. Soc. Jpn*. 5(1): 17-25.

Floyd, R., Abebe, E., Papert, A., and Blaxter, M. 2002. Molecular barcodes for soil nematode identification. *Mol. Ecol.* 11: 839–850.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial *cytochrome c oxidase subunit I* from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3(5): 294-299.

Gapud, V. P. 1981. Insect and mite pest of plant crops in Bangladesh and their natural enemies. In: *A Compendium for biological control in IPM*. United States Agency for International Development Agriculture Research Council/Chechi and Company Consulting Inc. 265pp.

Glover, R. H., Collins, D. W., Walsh, K., and Boonham, N. 2010. Assessment of loci for DNA barcoding in the genus *Thrips* (Thysanoptera: Thripidae). *Mol. Ecol. Resour*. 10(1): 51-59.

Gossner, M. and Hausmann, A. 2009. DNA barcoding enables the identification of caterpillars feeding on native and alien oak. *Mitt. Muench. Entomol. Ges*. 99: 135-140.

Gupta, S. K. 1985. *Handbook. Plant Mites of India*. Zoological Survey of India, Calcutta, 520p.

Gupta, S. K. 1992. Arachnida: Plant mites (Acari). In: Ghosh, A. K. (ed.), *Fauna of West Bengal* (3rd Ed.). Zoological Survey of India, Calcutta, pp. 61-211.

Gupta, S. K. 1995. Plant mites (Acari). In: Alfred, J. R. B. (ed.), *Fauna of Meghalaya* (2nd Ed.). Zoological Survey of India, Culcutta, pp. 17-50.

Gupta, S. K. and Chatterjee, K. 1997. Acari: plant mites. *Fauna of Delhi* (6th Ed.). Zoological Survey of India, Culcutta, pp. 485-532.

Gupta, S. K. and Gupta, Y. N. 1994. A taxonomic review of Indian Tetranychidae (Acari: Prostigamata) with description of new species, re-descriptions of known species and keys to genera and species. *Mem. Zool. Surv. India*.18: 1-196.

Haghghadam, Z. M. and Arbabi, M. 2012. Study of mites' fauna and their natural enemies on the ornamental plants in greenhouses of Guilan and West Mazandaran province. *Int. J. Agric. Crop Sci.* 4(11): 674-679.

Hajibabaei, M., Janzen, D. H., Burns, J. M., Hallwachs, W., and Hebert, P. D. N. 2005. DNA barcodes distinguish species of tropical Lepidoptera. *Proc. Natl Acad. Sci.* 103(4): 968-971.

Haque, M., Islam, T., Naher, N., and Haque, M. M. 2011. Seasonal abundance of spider mite *Tetranychus urticae* Koch on vegetable and ornamental plants in Rajshahi. *Univ. J. Zool., Rajshahi Univ.* 30: 37-40 available: http://journals.sfu.ca/bd/index.php/UJZRU.pdf. [14 July 2019].

Hebert, P. D. N., Ratnasingham, S., and. DeWaard, J. R. 2003a. Barcoding animal life: *cytochrome c oxidase subunit I* divergences among closely related species. *Proc. Roy. Soc. Biol. Sci.* 270: 96–99.

Hebert, P. D. N., Cywinska, A., Ball, S. L., and deWaard, J. R. 2003b. Biological identifications through DNA barcodes. *Proc. Roy. Soc. Biol. Sci*. 270: 313–321.

Henderson, R. C. 2001. Technique for the positional slide mounting of Acari. *Syst. Appl. Acarol*. 7: 1-14.

Hinomoto, N., Osakabe, M., Gotoh, T., and Takafuji, A. 2001. Phylogenetic analysis of green and red forms of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in Japan, based on mitochondrial *cytochrome oxidase subunit I* sequences. *Appl. Entomol. Zool.* 36(4): 459-464.

Hinomoto, N., Tran, D. P., Pham, A. T., Ngoc Le, T. B., Tajima, R., Ohashi, K., Osakabe, M., and Takafuji, A. 2009. Identification of spider mites (Acari: Tetranychidae) by DNA sequences: a case study in Northern Vietnam. *Int. J. Acarol.* 33(1): 53-60.

Hinomoto, N., Tran, D. P., Pham, A. T., Ngoc Le, T. B., Tajima, R., Ohashi, K., Osakabe, M., and Takafuji, A. 2007. Identification of spider mites (Acari: Tetranychidae) by DNA sequences: a case study in Northern Vietnam. *Int. J. Acarol.* 33(1): 53-60.

Hoy, M. A. 2011. *The Strategy of Integrated Mite Management. Agricultural Acarology: Introduction to Integrated Mite Management*. CRC Press, Boca Raton, USA, 410 p.

Inak, E., Alpkent, Y. N., Cobanoglu, S., Dermauw, W., and Leeuwen, V. T. 2019. National Library of Medicine (US), National Centre for Biotechnology Information [1988]- Accession No. MK508722, *Tetranychus urticae*, strain GSS *cytochrochrome oxidase subunit I* (*COI*) gene, partial cds; mitochondrial. [cited 2019 June 27]. Available from: https://www.ncbi.nlm.nih.gov/nuccore/MK508722.1.

[Indiaagristat]. 2019. Socio-economic statistical information about agriculture in India. [on-line]. Available: https://www.indiaagristat.com [25 August 2019].

Jagadish, K. S., Shadakshari, Y. G., Gowda, C. C., and Srinivasa, N. 2013. New record of spider mite, *Tetranychus ludeni* Zacher (Acari : Tetranychidae) on sunflower and reaction of few CMS lines to mite infestation. *Insect Environ.* 19(2): 106-107.

Jeppson, L. R., Keifer, H. H., and Baker, E. W. 1975. *Mites injurious to economic plants*. University of California Press, 614p.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., and Madden, T. L. 2008. NCBI BLAST: a better web interface. *Nucleic acids res.* 36(2): 5-9.

Karmakar, S. 2018. A survey on the diversity of mites inhabiting the ornamental and fruit plants in south Bengal, India. *Int. J. Adv. Res.* 6(8): 125-128. Available: http://dx.doi.org/10.21474/IJAR01/7498 .pdf. [15 March 2019].

Khot, K. N. and Patil, G. P. 1956. Life history of the gladiolus mite, *Tetranychus equatorius* McGregor. *Indian J. Sci.* 18(2): 149-164.

Krishnamurthy, P. K. and Francis, R. A. 2012. A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodivers. Conserv*. 21(8): 1901-1919.

Kumar, V., Kundu, S., Chakraborty, R., Sanyal, A., Raha, A., Sanyal, O., Ranjan, R., Pakrashi, A., Tyagi, K., and Chandra, K. 2018. DNA barcoding of Geometridae moths (Insecta: Lepidoptera): a preliminary effort from Namdapha National Park, Eastern Himalaya. *Mitochondrial DNA B* 4(1): 309-315.

Lee, M. L. and Lee, M. H. 1997. Amplified mitochondrial DNA identify four species of *Tetranychus* mites (Acarina: Tetranychidae) in Korea. *Kor. J. Appl. Entomol*. 36: 30-36.

Lekha, C. and Kinathi, S. 2019. Phytophagous mites infesting vegetable crops of Kerala, India. *Persian J. Acarol.* 8: 69–72.

Lenin, N. and Bhaskar, H. 2016. Mite pests of vegetable crops under protected cultivation in Kerala. *Entomon*, 41 (3): 305-308.

Lenin, N., Bhaskar, H., Abida, P. S., and Mathew, P. M. 2015. Molecular approach in species determination of *Tetranychus* complex in polyhouse cucumber. *Genome*, 58(5): 244.

Li, G. Q., Xue, X. F., Zhang, K. J., and Hong, X. Y. 2010. Identfication and molecular phylogeny of agriculturally important spider mites (Acari: Tetranychidae) based on mitochondrial and nuclear ribosomal DNA sequences, with an emphasis on *Tetranychus*. *Zootaxa*. 2647: 1-15.

Lowenstein, J. H., Amato, G., and Kolokotronis, S. O. 2009. The real *maccoyii*: identifying tuna sushi with DNA barcodes–contrasting characteristic attributes and genetic distances. *PloS one*, 4(11), p.e7866.

Manesh, K. B. 1972. Fauna of mites plant in Iran. Pests and Plant Diseases. *Iran J.* 35: 30-38.

Manson, D. C. M. 1963. Mites of the families Tetranychidae and Tenuipalpidae associated with citrus in South East Asia. *Acarologia*, 5: 351-364.

Matsuda, T., Fukumoto, C., Hinomoto, N., and Gotoh, T. 2013. DNA-based identification of spider mites: molecular evidence for cryptic species of the genus *Tetranychus* (Acari: Tetranychidae). *J. Econ. Entomol.* 106(1): 463-472.

Matsuda, T., Hinomoto, N., Singh, R. N., and Gotoh, T. 2012. Molecular-based identification and phylogeny of *Oligonychus* species (Acari: Tetranychidae). *J. Econ. Entomol.* 105(3): 1043-1050.

Mersino, E. 2002. *Mites on ornamentals*. Cooperative Extension Service, College of Tropical Agriculture and Human Resources, University of Hawai, 3p.

Metcalf, C. L. and Flint, W. P. 1962. *Destructive and Useful Insects, Their Habits and Control*. CABI pubishing, New York, 1087p.

Migeon, A. 2015. The Jean Gutierrez spider mite collection. *Zookeys*, 489: 15-24.

Migeon, A., Nouguier, E., and Dorkeld, F. 2010. Spider Mites Web: a comprehensive database for the Tetranychidae. *Trends Acarol*. 86: 557-560.

Monaghan, M. T., Balke, M., Gregory, T. R., and Vogler, A. P. 2005. DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Phil. Trans. R. Soc. Biol. Sci*. 360(1462): 1925-1933.

Mutanen, M., Aarvik, L., Huemer, P., Kaila, L., Karsholt, O., and Tuck, K. 2012. DNA barcodes reveal that the widespread European tortricid moth *Phalonidia manniana* (Lepidoptera: Tortricidae) is a mixture of two species. *Zootaxa* 3262(1): 1-21.

Mutisya, D. L., Ghimire, S. R., Njarui, D. M. G., Kago, L., Githae, D., and Mutai, C. 2018. Abundance and identity of red spider mite species on *Brachiaria*

grass in Kenya and its worldwide comparative phylogeny. *Can. J. Biotechnol*. 2(1): 100-107.

Nanney, D. L. 1982. Genes and phenes in *Tetrahymena*. *Bioscience 32*(10): 783-788.

Nassar, O. A. and Ghai, S. 1981. Taxonomic studies on tetranychoid mites infesting vegetable and fruit crops in Delhi and surrounding areas. *Orient. Insects*, 15: 333-396.

Natarajan, N. 2001. Screening of Amaranthus germplasms against Tetranychid mites. *Proceedings of CAS training on biodiversity of phytophagous mite and recent advances in their management*, p.185.

Navajas, M. and Fenton, B. 2000. The application of molecular markers in the study of diversity in acarology: a review. *Exp. Appl. Acarol*. 24: 751-774.

Navajas, M., Gutierrez, J., and Lagnel, J. 1996. Mitochondrial *cytochrome oxidase* I in tetranychid mites: a comparison between molecular phylogeny and changes of morphological and life history traits. *Bull. Entomol. Res.* 86: 407-417.

Navajas, M., Lagnel, J., Gutierrez, J., and Boursot, P. 1998. Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial *CO*I polymorphism. *Heredity*, 80: 742-752.

Navajas, M. 1998. Host plant associations in the spider mite *Tetranychus urticae* (Acari: Tetranychidae): insights from molecular phylogeography. *Exp. Appl. Acarol*. 22: 201–214.

Onkarappa, S., Mallik, B., and Harishkumar, M. 1999. Spatial distribution of *Tetranychus urticae* Koch on open cultivated rose. *J. Acarol*. 15: 44-46.

Packer, L., Grixti, J. C., Roughley, R. E., and Hanner, R. 2009. The status of taxonomy in Canada and the impact of DNA barcoding. *Can. J. Zool*. 87(12): 1097-1110.

Pal, S. and Sarkar, I. 2009. Pests infesting ornamental plants in hilly region of West Bengal. *Int. J. Plant Prot.* 1(1): 98-101.

Pirone, P. P. 1978. *Diseases and Pests of Ornamental Plants*. John Wiley & Sons. 584 p.

Post, R. J., Flook, P. K., and Miller, A. L. 1993. Methods for the preservation of insects for DNA studies. *Biochem. Syst. Ecol.* 21: 85-92.

Pritchard, A. E. and Baker, E. W. 1955. *A revision of the spider mite family Tetranychidae*. Mem. Pac. Coast. Entomolo. Soc. 472pp.

Raja, M. and Perumal, P. 2017. DNA Barcoding and phylogenetic relationships of selected south Indian freshwater fishes based on mtDNA *COI* sequences. *J. Phylogenetics Evol. Biol.* 5(2): 184.

Rather, A. Q. 1983. New records of five genera and eighteen species of phytophagous mites (Acarina) from India with notes on their host range, distribution and economic importance. In: Abstracts of 2nd All India Symposium on Acarology, Pune, 1983, pp. 25-26.

Rebijith, K. B., Asokan, R., Kumar, N. K., Srikumar, K. K., Ramamurthy, V. V., and Bhat, P. S. 2012. DNA barcoding and development of species-specific markers for the identification of tea mosquito bugs (Miridae: Heteroptera) in India. *Environ. Entomol.* 41(5): 1239-1245.

Reid, B. N., Le, M., McCord, W. P., Iverson, J. B., Georges, A., Bergmann, T., Amato, G., Desalle, R., and Maciel, E. N. 2011. Comparing and combining distance based and character based approaches for barcoding turtles. *Mol. Ecol. Resour.* 11(6): 956-967.

Rogers, S. O. and Bendich, A. J. 1994. Extraction of total cellular DNA from plants, algae and fungi. *Plant Mol. Biol. Manual* 1: 1-8.

Ros, V. I. D. and Breeuwer, J. A. J. 2007. Spider mite (Acari: Tetranychidae) mitochondrial *COI* phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. *Exp. Appl. Acarol*. 42(4): 239-262.

Sadurudeen, N., Kumar, A. P., Babu, P. G., Jaiswar, A. K., Chaudhari, A., Krishna, G., and Lakra, W. S. 2017. DNA barcoding of selected Perciformes (Infra Class: Teleostei) fishes from Indian coast. *Indian J. Biotechnol.* 16(3): 315-321.

Shah, D. R. and Shukla, A. 2014. Seasonal incidence of spider mite, *Tetranychus urticae* Koch (Tetranychidae: Acari) on *Gerbera* (*Gerbera jamesonii*) under polyhouse conditions. *Pest Manag. Hort. Ecosyst.* 20(1): 26-29.

Sharma, N. D. 2005. Biology and control of insect and mite pests of rose (First Red) under polyhouse conditions. M.Sc.(Ag) thesis, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, 47p.

Simon, C., Frati, F., Veckenvach, A., Crespi, V., Liu, H., and Flook, P. 1994. Evolution, weighing and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymer chains reactions primers. *Ann. Entomol. Soc. Am.* 87(6): 651-701.

Sepasgozarian, H. 1971. Mites and their economic importance in Iran. In: Danie, M. (ed.), *Proceedings of the 3rd International Congress of Acarology, Prague,* Plant Medicine Department, Agricultural College of Tehran University, pp. 241-242.

Singh, V. 2015. Phytophagous mites and their natural enemies in different horticultural ecosystems of Himachal Pradesh. PhD thesis, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, 169p.

Singh, J. and Raghuraman, M. 2011. Emerging scenario of important mite pests in north India. *Zoosymposia* 6: 170–179.

Singh, V. and Chauhan, U. 2014. Diversity of mite (Acari) fauna associated with vegetables and ornamental plants in mid-hill conditions of Himachal Pradesh, India. *J. Biol. Control* 28: 75-80.

Smith, M. A., Wood, D. M., Janzen, D. H., Hallwachs, W., and Hebert, P. D. N. 2006. DNA barcodes affirm that 16 species of apparently generalist tropical parasitoid flies (Diptera, Tachinidae) are not all generalists. *Proc. Natl Acad. Sci.* 104(12): 4967-4972.

Sood, A. K. and Kakar, K. L. 1990. Record of insect and non-insect pests of ornamental plants from Himachal Pradesh. *J. Insect Sci.* 3(2): 141-145.

[Spider mites web]. 2019. A comprehensive database for the Tetranychidae. [on-line]. Available: http://www.montpellier.inra.fr/CBGP/spmweb [10 march 2019].

Srinivasa, N., Gowda, C. C., Mallik, B., and Raghavendra, P. 2012. New record of *Tetranychus truncatus* (Ehara) as a potential pest from Karnataka. *Indian J. Entomol*. 74(4): 379-383.

Srinivasa, N., Kavya, M. K., and Gowda, C. C. 2014. *Tetranychus turkestani* an invasive spider mite pest of grapevine in Karnataka and Maharashtra. Ramamurthy, V.V. and Subramanian, S., Entomology as a science and IPM as a technology - the way forward, Pasighat, India: 61-62.

Stoeckle, M. 2003. Taxonomy, DNA, and the barcode of life. *BioSci.* 53 (9): 796-797.

Sudharma, K. and Nair, G. M. 1999. Occurrence of phytophagous mites and natural enemies on vegetables in Kerala. *Insect Environ*. 5: 15-16.

Taylor, H. R. and Harris, W. E. 2012. An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. *Mol. Ecol. Resour*. 12(3): 377-388.

Tehri, K. 2014. A review on reproductive strategies in two spotted spider mite, *Tetranychus urticae* Koch 1836 (Acari: Tetranychidae). *J. Entomol. Zool. Studies*, 2(5): 35-19.

Trewick, S. A. 2000. Mitochondrial DNA sequences support allozyme evidence for cryptic radiation of New Zealand *Peripatoides* (Onychophora). *Mol. Ecol*. 9: 269–281.

Valentini, A., Miquel, C., Nawaz, M. A., Bellemain, E. V. A., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P., and Swenson, J. E. 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *trn*L approach. *Mol. Ecol. Resour*. 9(1): 51-60.

Van de Vrie, M., Helle, W., and Sabelis, M. W. 1985. *Spider Mites: Their Biology, Natural Enemies and Control*. Elsevier Amsterdam, Netherlands pp. 273-283.

Vogler, A. P. and Monaghan, M. T. 2007. Recent advances in DNA taxonomy. *J. Zool. Syst. Evol. Res.* 45(1): 1-10.

Zeity, M., Srinivasa, N., and Gowda, C. C. 2016. New records of spider mites and description of male of *Stylophoronychus baghensis* Prasad (Acari: Tetranychidae) from India. *Orient. Insects* 50(3): 119-128.

Zeity, M., Srinivasa, N., and Gowda, C. C. 2016. New species, new records and re-description of spider mites (Acari: Tetranychidae) from India. *Zootaxa*, 4085 (3): 416–430.

Zhang, Z. Q. 2003. *Mites of Greenhouses: Identification, Biology and Control*. CABI Publishing, Wallingford, 244 p.

Annexure

ANNEXURE I

1. Materials used in collection and rearing of spider mites

Transparent polythene bags

Rubber Bands

Artist Brush (0 Size)

Polyethylene trays (Small)

Absorbent sponge

2. Materials used in permanent slide preparation

Hoyer's medium

Slides

Cover slip

Syringe

3. Composition of Hoyer's medium

Sl. No.	Content	Quantity
1	Chloral hydrate	200 g
2	Gum arabic	30 g
3	Glycerol	20 ml
4	Distilled water	50 ml

ANNEXURE II

Reagents used for DNA isolation

1. **CTAB extraction buffer (100 ml)**

CTAB (Cetyl Trimethyl ammonium bromide)	: 2 g
Tris HCl (1M, pH- 8)	: 10 ml
EDTA (0.5 M, pH- 8)	: 2 ml
NaCl (5M)	: 30 ml
Distilled water	: 54 ml

2. **Chloroform: Isoamyl alcohol (24: 1 v/v)**

To twenty four parts of chloroform, one part of isoamyl alcohol was added and mixed properly.

3. **Sodium acetate (3M)**

40.8 g of sodium acetate put in 100ml distilled water and mixed well. The mixture was stored in refrigerator at 4° C.

4. **Ethanol (70%)**

To seventy parts of absolute ethanol thirty parts of distilled water was added and mixed well. This 70 per cent alcohol was stored at 4° C.

ANNEXURE III

Composition of buffer and dyes used for gel electrophoresis

1. TAE buffer (50X)

Tris base : 242g

Glacial acetic acid : 57.1ml

0.5M EDTA (pH- 8) : 100ml

2. Loading dye (6X)

0.25% Bromophenol blue

0.25% Xylene cyanol

30% Glycerol in water

3. Ethidium Bromide

The dye was prepared as a stock solution of Ethidium bromide (stock 10mg/ml; working concentration 0.5ug/ml (Genie)) and was stored at room temperature in a dark bottle.

4. Agarose gel

Gel was prepared with 1.2 per cent agarose for assessing PCR samples.

ANNEXURE IV

List of *COI* sequences obtained from different spider mite species in the study

>Cel1vk
GAAATTGATTAGTTCCCTTAATAGTTAACACTGTAGATTTATGTTTTGCACGAATTAATA
ATATAAGATTTTGACTATTAATTCCTTCTCTTATCTTAATAATTAGTTCTTCCATAAAAAG
AGTTATAAATGGAGTTGGATGAACAATATATCCTCCATTAACTTCAATTCAATATTTTAT
ATCTTCTTCTATTGAAATAATAATTTTTTCTTTACATGTTGCAGAATTTCATCAATTGCTA
GATCTATTAATTTTATTTCAACTATTTTATTAATAAAAAATAAAAACTATTATTTAAGTAA
TTTAACATTGTTTTCTTTATCAATTTTAATTACTACTTTATTACTTTTATTAGCTTTACCTG
TATTAGCAGGTGCTATTACAATAATTTTAATAGATCGAAACTTTAATACATCATTTTTTG
ATCCTAGAGGAGGAGGAGACCCAATTTTATATCAACATTTATTCTGATTTTTTGGGCATC
CAGAAGTTTATATTTTAATTTTACCAGGTTTTGGAATGATTTCACACATTATTAGATATA
ATTTAGGTAAAAAAGAAGTTTTTGGTAAAATTGGAATAATATTTGCTATAATATCGATT
GGTTTATTAGGTTTTATTGTATGAGCTCACCACATATTTACAGTAGGAATAGATGTTGA
TACACGAGCTTACTTTACTGCTGCTACAATAATTATTGCTATTCCTACTGGAATTAAAA
TTTTTAGTTGATTTACTACAATTTTAAATTCACATATTAATTTTAATATTTCTATATATTG
ATCTATAGGATTTTTAATTATATTTTCTATTGGAGGATTTACAGGAATTGTAGCTTCAAA
TTCATGTTTGGATATTAATTTACATGACTCATATTATATTG

> Cel2vk

TTAACAATATAAGATTTTGACTTTTAATTCCTCTATTATTCTTATAATTAGAGCTTCAATA
AAAAGAGTAATAAATGGGGTAGGATGAACAATATATCCTCCTTTAACTTCAATTCAATTT
TATATATCTTCATCCATTGAAATAATAATTTTTTCTCTTCATATTGCAGGGTTATCATCTAT
TGCTAGTTCTATTAATTTTATTTCAACTATTTTATTAATAAAAAATAAAAATTATATTTTT
AGAAGTTTAACTTTATTTTCTTTATCAATTTTAATTACTACCTTTTTACTTTTACTAGCTTT
ACCAGTATTAGCAGGTGCTATTACAATAGTTTTAATAGATCGAAATTTTAACACGTCTTTT
TTTGATCCTAGAGGAGGGGGAGATCCTATTTTGTATCAACATTTATTTTGATTTTTTGGTC
ATCCAGAGGTTTATATTTTAATCTTACCAGGTTTTGGAATAGTTTCACATATTATTAGATA
TAATTTAGGAAAAAAAGAAGTGTTTGGAAAAGTTGGAATATTATTTGCTATAATATCAAT
TGGATTATTAGGATTTATTGTATGAGCTCATCATATATTTACAGTAGGAATAGATGTAGA
TACTCGAGCTTATTTTACAGCTGCTACTATAATTATTGCAATTCCTACTGAATTAAAATTT
TTAGTTGATTTACAACTATTATTAATTCACATATTAATTTTAATACATCAATATACTGATC
TATAGGATTTTTAATTATGTTTTCAATTGGAGGATTTAC

>Chr2mk
TAAAAAGTGTTTTAAATCGAGTGGGGTTGAACAATATATCCCCCCCTAACTTTCAAATTC
AATATTTTATGTCTTCCTCTATTGAAATAATAATTTTTTCTTTACATATTTGCAGGAATTTC
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Abstract

DNA BARCODING OF SPIDER MITES (PROSTIGMATA: TETRANYCHIDAE) ASSOCIATED WITH ORNAMENTAL PLANTS

by

JAYALAKSHMI PRAKASH

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Abstract of the thesis

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ABSTRACT

Total area under floriculture in India is estimated to be 0.31 million hectares with a production of 2.08 million tonnes loose flowers and 0.82 million tonnes cut flowers in 2018-19. In Kerala, Thrissur district is considered as the centre of floriculture nursery business. Majority of nurseries in the area do not maintain their own sources of mother plants and hence import planting materials from other countries. Trade of commercial ornamentals has been recognized worldwide as an important invasion pathway for non-native pests, especially insects and mites. Though mites are reported as major pests of commercial ornamental plants from different parts of India, no systematic study has been conducted so far to document the diversity of mites associated with ornamental plants in Kerala. Considering this, the present study, 'DNA barcoding of spider mites (Prostigmata: Tetranychidae) associated with ornamental plants' was undertaken with the objectives to generate DNA barcodes for different species of spider mites infesting commercial ornamental plants of central Kerala and to find the genetic variability among them.

The study included collection and culturing of spider mites, morphology based identification and molecular characterization of selected accessions. Purposive sampling surveys were carried out in commercial ornamental nurseries and homestead gardens of Thrissur and Ernakulam districts, covering 12 ornamental plants. Mite infested leaf samples were collected, brought to the laboratory and maintained separately as isoline cultures by assigning unique accession numbers.

Morphological characterization of 26 isoline cultures revealed the occurrence of six species of spider mites under two genera *viz*., *Tetranychus* and *Oligonychus*. The genus *Tetranychus* was more diverse with five species *viz*., *Tetranychus truncatus, T. urticae, T. okinawanus, T. neocaledonicus* and *T. marianae.* The genus *Oligonychus* was represented by only one species, *Oligonychus biharensis* Hirst. Rose recorded the highest diversity of spider mites with five species. The mite species, *T. okinawanus*

recorded wider host range with eight host plants *viz*., *Adenium*, rose, *Gerbera*, chrysanthemum, orchid, cairo morning glory, marigold and balsam. All the host plants except *Adenium* are new host records of *T. okinawanus* from India. The study recorded three new host plants for *T. truncatus* from India *viz.*, rose, cock's comb and marigold. In this study, *T. marianae* was recorded for the first time from Kerala.

For molecular characterization, DNA was isolated and *COI* locus of 868 bp length was amplified using universal primer, specific to *COI*. Polymerase chain reaction (PCR) products of 12 accessions representing five species were sequenced and *in-silico* analysis was carried out. Homology analysis of sequences of 11 accessions showed 90-99 per cent similarity with sequences in NCBI database, which were in consensus with morphological identification. The sequence of the accession, Ros1vk (*T. marianae*) did not show significant similarity with any of the sequences in the NCBI database. In this study, *COI* sequence of *T. marianae* was submitted for the first time in GenBank.

Barcode gaps among the species were examined by aligning the *COI* sequences using Clustal Omega tool and species-specific barcodes were identified at different nucleotide positions. Pairwise distance analysis of the sequences showed intraspecific divergence ranging from 0.00 to 0.036 and interspecific divergence ranging from 0.070-0.217. Phylogenetic analysis revealed the monophyly of *T. truncatus* and *T. urticae* and the close relationship of *T. marianae* with *T. okinawanus*.

The study has shown that rose harbours many species of spider mites, indicating the need for imposing strict quarantine regulations for movement of planting materials of rose to avoid entry and invasion of mites into newer areas. The potential of *T. okinawanus* and *T. truncatus* to turn invasive in Kerala's ecosystems is also brought out. The study establishes the reliability of *COI* locus as a marker for species discrimination in spider mites.