DNA BARCODING OF THRIPS (THYSANOPTERA: THRIPIDAE) ASSOCIATED WITH SELECTED VEGETABLE CROPS OF KERALA

by

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(2015-09-023)

THESIS

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requirements for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**DNA BARCODING OF THRIPS (THYSANOPTERA: THRIPIDAE) ASSOCIATED WITH SELECTED VEGETABLE CROPS OF KERALA."** 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, associate ship, fellowship or other similar title, of any other university or society.

Place: Vellayani
Date:

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Namitha T. H.

DEDICATED TO MY PARENTS,
TEACHERS AND FRIENDS

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CERTIFICATE

Certified that this thesis entitled "**DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala**" is a record of research work done independently by Ms. Namitha T. H. 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. Namitha T. H. (2015-09-023) a candidate for the degree of **B. Sc. – M. Sc. (Integrated) Biotechnology,** agree that the thesis entitled "**DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala**" may be submitted by Ms. Namitha T. H., in partial fulfilment of the requirement for the degree.

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1. INTRODUCTION

The family Thripidae insects are also known as thrips which regarded as the most notorious pest among vegetable crops. Thrips are minute insects in the size ranging from 0.5-1.5 millimetres in length, adults are longer, slender and have long fringes on the margins of both pairs of their leaned long wings. Younger ones are delicate, elongated and wings are absent. Colour range of majority are from crystalline white or yellowish to dark brown or black.

Most species have been recorded as pest, predator and plant virus transmitter (Lewis 1997, Pappu *et al*., 2009). As most of them causes direct damage to crops, a few act as Tospovirus transmitter (genus Tospovirus, which could make heavy impact on vegetable, fiber and ornamental crops by spreading viral diseases (Whitfield *et al.,* 2005).

Morphological characteristics encompassing color, antennal segments, body setae, wing setae, and presence or absence of a comb on the VIII abdominal segment were frequently utilized as taxonomic tools for thrips identification. In spite of that, morphological identification found difficult due to their tiny size, shortage of rigid morphological characters, high cryptic appearance, sexual dimorphisms, overlapping host ranges and the requirement of taxonomic expertise (Brunner *et al*., 2002, Murai *et al*., 2001). As there are no sufficient keys for identification of egg, larvae, or pupae leads to the limitation of morphological examination to adult specimens (Kadirvel *et al*., 2013). Molecular identification of species could be the most acceptable strategy which were inexpensive, rapid, appropriate to wide range screening and does not require morphological expertise (Rubinoff *et al*., 2006). Several molecular markers are accessible today for species determination including several nuclear genes (18S rRNA and 28S rRNA) and internal transcribed spacers (rDNA ITSs), as well as the mitochondrial cytochrome c oxidase I (mt COI) gene (Barr *et al*., 2005).

Novel molecular biology tools to an extent helps in identification of life forms in precise and easy manner. DNA barcoding regarded as the most desirable technique to detect known and unknown species based on nucleotide alignment in

DNA fragment (Novotny *et al*., 2002). DNA barcoding is the use of short standardized DNA sequence of 658 bp fragment of the mitochondrial cytochrome c oxidase (COI) gene to identify and assign unknown specimens to species besides facilitating the discovery of new species.

Variations of DNA sequences in mitochondrial and nuclear segments were widely used for insect taxon determination via molecular methods. Differentiation of thrips species can be attained from the sequences of mitochondrial cytochrome oxidase I (mt COI) coding gene from different species (Brunner *et al*., 2002). BLAST analysis of barcode sequences of any species could help to disclose the identity, species relationship with the existing organisms from the database.

Thrips identification is essential for an efficient integrated pest management programme through DNA barcoding, the current study was undertaken with the objective to generate DNA barcodes for different species of thrips infesting selected vegetable crops of Kerala and to find out the variability among them.

2. REVIEW OF LITERATURE

The study regarding "DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala" has been performed through the amplification of mitochondrial cytochrome oxidase subunit I (COI) from various species belonging to Thripidae family. The amplified COI gene fragments were sequenced and the information were utilised to detect the relationship within and between species. The pertinent literatures which accessible on different aspects on the study were collected and reviewed in this chapter, under various headings.

2.1. Thrips taxonomy

Scientific classification of thrips:

Kingdom : Animalia

Phylum : Arthropoda

Class : Insecta

Order : Thysanoptera

Family : Thripidae

Species : Thrips

2.2. Identification of thrips

Accurate level identification of species provides precise dataset on species diversity, phylogenetic patterns, and evolutionary processes. Morphological based identification of thrips found too strenuous process due to their tiny size, lack of morphological differentiation, intraspecific polymorphisms, and sexual dimorphisms (Tyagi *et al*., 2008). As it is regarded as one of the notorious pest causing widespread damages in vegetables, fiber and ornamental crops by functioning as predator, pest, and plant virus transmitter (Tospoviruses) (Whitfield *et al*., 2005). Cryptic behaviour strategy of thrips cause misleading morphological

identification, this turns into effective pest management strenuous. In cryptic pest species, accurate identification is extremely important for quarantine and for developing biological and other control strategies.

Morphological identification of species seems to be a tedious process which requires taxonomical experts, microscopic slide preparations and comprehending taxonomic features which always happened to be applicable only at adult stage. Following limitations can be solved by molecular based identification which are functioning in an effortless manner with high level of accuracy. Another important feature is any non-taxonomist can identify species and species level relationship. Molecular level approaches in taxon detection exploits variations in DNA segments to recognize all life forms (Kurtzman *et al*., 1994). The most popular easy and convenient globally accepted molecular method for species level identification is DNA barcoding which exhibiting mitochondrial and nuclear DNA sequencing for taxon identification (Hebert *et al*., 2005)

DNA polymorphisms in mitochondrial and nuclear genes has efficiently employed in insect taxon determination (Simon *et al*., 1994). COI sequences from different thrips species were used to detect various haplotypes (Brunner *et al*., 2002). Rather than COI, several PCR based molecular markers were evolved with the utilization of nuclear ribosomal DNA sequences, internal transcribed spacer 1 and 2 (ITS2) for establishing the variations in thrips (Rugman *et al*., 2006).

Various molecular markers have been developed from rRNA encoding genes comprising 18S rRNA, 28S rRNA and rDNA internal transcribed spacers several nuclear genes for the utilization of DNA barcoding.

2.3. Morphological characters of thripidae

Genus thrips is generally characterized by three autapomorphies, the derived traits which are unique to thrips taxon: absence of ocellar setae I, abdominal tergites V–VIII with paired ctenidia laterally, tergite VIII with ctenidia posteromesad to the spiracles (Mound, 2002).

Thrips generally classified into two suborders with a few distinctive characteristics; the Terebrantia, with a blunt or angled body end, and the Tubulifera, in which the abdomen forms a tube at the end (Moritz, 1994; Moritz *et al*., 2000). Adults and larvae of both suborders, Terebrantia and Tubulifera, share one unique structural attribute: Among the two mandibles, left attained full development, and the right is adhered by embryo (Heming *et al*.,1993)

In Greek Thysanoptera meant by "trimmed wings," as both pairs of wings in adults posses a marginal fringe of long cilia, inspite of wings with fringes occur in several other groups of small insects and adults of many thrips are wingless.

Thrips usually seen in a size range of 1.5 to 3 mm in length, with the smallest possess 0.6 mm and the largest are in 15 mm. Due to their tiny size, they could attack smaller flowers or little spots in stems, leaves and bark.

2.3.1. External morphology of thrips

Head consists of antennae, eye and mouth parts

Antennae

- Antennae relatively shorter in appearance
- 4 to 9 segmented antennae
- Bead like (monoliform) or filiform.
- The family of thrips distinguished greatly by the characters of antennae, especially by the number of antennal segment and the nature of sensoria on the third and fourth segments.

Mouth parts

- Rasping and sucking mouth parts
- Distinct assymetrical mouth parts
- Lower right mandible and vestigial and in some species completely absent.
- Left mandible appears larger and raise a thin stylet used to pierce the tissue cellwall.
- Some species could inject digest enzymes through the maxillary stylets and hypopharynx could loaded into the openings to drain cellular fluids.These process causes a unique silvery or bronze scarring on the surfaces of the stems and leaves where the thrips feed.

Eyes

- Thrips have compound eyes.

Wings

- 2 pairs
- Strippy type
- Wings are modified into strips or rod like structures and fringed with long hair
- Colour varied accordingly to the type of species

2.4. Biology of thrips

Thrips are small, phytophagous opportunistic, vagile, and ubiquitous insects which are adapted to be invasive to host species and generally occurs as yellow, brown, or black in color. Their morphological appearance can exceeds from nondescriptive to species with established secondary sexual characteristics. Eggs laid either inside or outside of the host plants. Metamorphosis is complicated with two larval instars, a propupal stage, and up to two pupal stages, depending on the two suborders.

Terebrantia and Tubulifera are the two suborders of thrips in which the former one always lay eggs. Ovoviparity most often found in highly evoluted members of Tubulifera. Eggs usually found as elongated, oval to kidney shaped and slightly sculptured. Eggs of suborder Terebrantia often placed into alive plant cells in a tear produced by the female ovipositor. In spite of that, eggs of Tubulifera, whose females devoid of ovipositor hide beneath the bark, inside or within flowers and leaves or attached within bark. Eggs most often took 4 to 15 days to hatch with respect to the species and environmental conditions.

The larvae of thrips are miniature and simpler in structure. Longer anal bristles feature of larvae could hold droplets of excreted liquid and digesting enzymes that were settled on leaf surfaces. Thrips damage on plants could be identified through discrete dark spots of excreta near to pale coloured feeding zones of leaves.

The initial stage of larvae is too short, limits only for one or two days. The next one could exceeds to three to four months when estivation or hibernation period occurs. The subsequent stage of Terebrantia forms into a prepupa, but in suborder Tubulifera in the beginning, a stage called primipupa evolved and is abided by the prepupal larvae.The final stage of pupa attained from prepupal larvae of thrips instantly and the last stage could be short or long period depending on the type of species. The pupation occurs in cocoons in case of Terebrantia and also on host plant in Tubulifera.

Insect biologists have sought unsuccessful in predicting it's invasive likelihood. They were generally enormous in place of origin, exhibiting faster migration and closely associated with human activities, which leads to it's widespread in all crops in the world and gradually adapted it's life with new condition. They often lack obligate diapause and are polyphagous. Some exhibits high abundance and shorter life cycle, parthenogenesis often happened due to short lifespan of males. These characteristics enhances it's resistance to insecticides and other pest management programmes (Morse *et al*., 2006).

A few thrips are devoid of wings, but this limitation doesn't alleviate invasiveness curve in their lifecycle. *Aptinothrips rufus* regarded as the best-known example for invasion by absence of wings and ubiquitious in whole world (Mound *et al*., 2004).

T. hawaiiensis were allocated to group V, due to the presence of abdominal sternites III-VII with discal setae, pleurotergites devoid of discal setae and metanotum usually striate or with some median reticulations (Morgan, 1913).

2.5. Feeding behaviour of thrips

Feeding mechanism of thrips is diverse because of feeding diverse crops. Most commonly observed feeding mechanism of thrips is leaf-feeding by piercing the surface cells of leaves with a single mandibular stylet and by inserting the paired maxillary stylets. It uses maxillary stylets for piercing inside the leaves for feeding

purpose (Heming *et al*., 1978). *Frankliniella schultzei*, *T. palmi* were regarded as notorious plant pests attacking leaves and flowers of host plants. Among these *T. palmi* is the crucial one which infesting cucurbits family.

Thrips are polyphagous insects with wide range of host crops which leads quarantine procedures highly hectic. Food scale varies based on the habitat of thrips. In plants, thrips used to feed plant leaf cells (Chisholm *et al*., 1983), petal, pollen, style cells, stamen (Bournier *et al*., 1979), seeds (Doull, 1956) and fruit (Lewis, 1973).

Thrips usually regarded as opportunistic species which adapted to exploit newly available habitats or resources and are typically found in unpredictable, transient, and variable environments. Predatory behaviour mostly observed in *Karnyothrips* species, attacks immature insects, whiteflies and mites (Houston *et al*., 1991). A few ones feed within their species group. These characteristics are related in their morphological behaviour, for eg; *Aeolothripids* are comparatively larger and migrate with very high speed which is regarded as a prime adaptation for predation (Mound, 2002).

2.6. Diversity of thrips

Thrips are highly invasive crop pest with high adaptive capacity and polyphagous behaviour. Adaptive characteristic of thrips is mostly observed in their feeding sources, variation in their life stages including variation in length of larval life, body size at pupation, pupation site, and threshold temperatures for development.

Chilli thrips prefers young leaves, buds and fruits of it's host plant and causes color variation from bronze to black in color. Chilli thrips found enormous during dry season (Seal *et al.*, 2005).

Ananthakrishnan *et al*. (1966) in earlier classified *T. hawaiiensis* under *T.florum* because of the confusing taxonomic characters shared by both species. Nakahara *et al*. (1985) discriminated these two predominantly on the presence of

sculpture around the anterior part on mesonotum known in hawaiiensis but the sculpture devoid in case of *T.florum.*

Rosenheim *et al*. (1990) reported *Thrips palmi* as a primary foliage feeder commonly occurs with a second thrips species, the western flower thrips, *Franklinella occidentalis* associated with two types of damages; direct damage caused by feeding on developing fruit and indirect damage caused by feeding on foliage.

2.7. Damages caused by thrips

Thrips are highly invasive opportunistic insects occupies contrasting niches resulting in a diverse array of lifestyles by the utilization of diverse resources.

Taeniothrips inconsequens attacks host plants at very early stages on the growing leaves, blooming flowers, emerging fruit and causes severe damages to the host plant. Beneficiary minute size and morphological characters enables them to enter highly complicated regions of plants including enclosed flowers. These type of feeding injuries in some plants could cause termination of plants permanently.

T. palmi is a serious pest causing acute damages to the economical vegetable crops like pepper, potato, tobacco, cucumber, melons, squash, cowpea and eggplant which have been reported from Asia (Sakimura *et al*., 1986).

Rosenheim *et al*. (1990) investigated direct feeding damage on cucurbitis by mixed species infections of *T. palmi* and *F. occidentalis*. He observed *T. palmi* infections mostly prevailed on foliage and *F. occidentalis* occurred highly on flowers, this makes them easily to feed and scar young fruits. Johnson *et al*. (1986) reported that silver colour appearance on the leaves of watermelon is due to *T. palmi* infestation.

In a study conducted to find out the interconnection of sugar maple budburst phenology with pear thrips infection by introducing five adult thrips to each bud on caged seedlings had observed reduction in total leaf area and average leaf size on

infested seedlings compared with the controls**.** Thrips infection which leaded to chlorosis, moisture less condition, thin canopy foliage. Thrips activity was highly observed on early-breaking than late-breaking buds.

2.8. Concept of DNA barcoding

DNA barcoding is a novel molecular based method designed to provide rapid and accurate species identifications by using short, standardized gene regions as internal species tags (Herbert *et al*., 2003). As an advantage, it will make the Linnaean taxonomic system more accessible, with benefits to ecologists, conservationists, and the diversity of agencies functioning with the control of pests, invasive species, and food safety. DNA barcoding allows more systematic analysis of organisms in the universe, by providing an easy access to the names and biological attributes of any species on the universe. Species with morphological identification difficult by taxonomists due to lack of characteristic features could assigning specimens to known species with the help of molecular level identification. Specific barcodes assigned for each species allows a non-taxonomists to rapidly sort specimens and by highlighting divergent taxa that may represent new species. By augmenting their capabilities in these ways, DNA barcoding offers taxonomists and non-taxonomists the opportunity to maximum enhance and eventually contribute to the unveiling of life's diversity. The 648- bp cytochrome c oxidase subunit I (COI) gene region used as the DNA barcode standard for members of the animal kingdom represents a complex composite character involving hundreds of independently varying components.

Tyagi *et al*. (2017) reported the largest DNA barcoding initiative on 370 sequences of 89 thrips morphospecies including 104 novel sequences from 39 morphospecies and utilized the multiple delimitation methods for detection and identification of cryptic species.

Chakraborty *et al*. (2019) reported the barcoding of highly polyphagous thrips species, *Scirtothrips* from India and contributed six sequences of three species (*S. hitam, S. mangiferae*, and *S. malayensis*) are the novel contribution in global database.

2.9. DNA isolation of thrips

Genomic DNA extraction from single thrips specimen is essential to avoid mixing up of genotypes and it is a challenging task because of it's minute size. Hence, researchers introduced several advantageous protocols for DNA isolation of thrips.

Rugman *et al*. (2010) utilized salting out procedure for DNA extraction of Thrips in which individual wingless adults were crushed in a 1.5mL microfuge tube and incubated at 37°C in 300 p,L TNES (50 mM Tris, pH 7.5, 400 mM NaCl, 20 mM EDTA, 0.5% SDS) with 100 pg/mL Proteinase K. After 3-18 h, proteins were precipitated with 5M NaCl and vortexing for 15 sec. After centrifugation of 14,000 rpm for 5 minutes results in pellet formation of proteins and DNA was precipitated from the decanted supernatant with 1 volume 100% ethanol. DNA was pelleted, washed in 70% ethanol, air-dried, and dissolved in sterile water.

Tyagi *et al*. (2008) used individual specimen of each species of Thrips by employing a non-destructive method of DNA extraction using QIAamp DNA Mini Kit (Qiagen, Valencia, CA) by making an intersegmental abdominal cut to each specimen, and these were lysed overnight at 56 °C in buffer ATL with proteinase K. Non-destructive method of DNA

extraction is regarded as the most easy, convenient and accurate method of insect DNA isolation.

2.10. COI locus

Mitochondrial genome of insects are better target for phylogenetic analysis than the nuclear genome because of its lack of introns, its limited exposure to recombination and its haploid mode of inheritance (Saccone *et al*. 1999). Universal primers for this gene are highly robust and possess greater range of phylogenetic signal than any other mitochondrial gene (Folmer *et al*., 1994; Zhang & Hewitt, 1997). Glover *et al*. (2010) claimed mitochondrial COI is the most suitable molecular

marker for species level identification within the genus thrips, because it exhibits more consistent interspecific variation than other markers and investigated the ability of five different loci of mitochondrial COI to discriminate thrips species.

Cytochrome oxidase I is associated in both electron transport and the related translocation of protons throughout the membrane and has been shown to contain a selection of distinct kinds of functional domain including ligand locations, ion channel elements, structural helices and interspersing hydrophilic loops (Saraste, 1990). Amino acid residues in strongly conserved reaction sites do not control the entire COI molecule, enabling for significant variation in some areas. Such a mixture of extremely preserved and variable areas so tightly connected with a mitochondrial gene makes the COI gene especially beneficial for evolutionary research

Crespi *et al*. (1998) employed nuclear 18S and mitochondrial gene cytochrome oxidase I (COI) genes to examine phylogenetic relationships between two suborders Terebrantia and Tubulifera. DNA barcoding via COI analysis has helped to reveal the number of thrips species inhabiting particular cropping systems (Kadrivel *et al*., 2013).

The Barcode Index Number (BIN) system now provides an interim taxonomic system based on COI sequence clusters for all animals and most BINs are congruent with morphological species (Ratnasingham *et al.*, 2003).

Macharia *et al*. (2015) reported thrips as the primary vectors of tomato spotted wilt virus (TSWV) with *Frankliniella occidentalis* (Pergande) by exploiting cytochrome oxidase I (COI) gene to establish potential vector populations within thrips.

2.11. ITS2 locus

Ribosomal DNA is comprised of multiple copies of tandemly repeated transcriptional units, each consisting of a conserved transcribed region, including genes that code for the ribosomal subunits 18S, 5.8S, and 28S, and two internal transcribed spacers (ITS1 and ITS2). Each unit is separated by a variable intergenic spacer (IGS) (Beckingham *et al*., 1982).

The internal transcribed spacer 2 (ITS2) of the nuclear rDNA cistron is a widely used phylogenetic marker. Kumar et al. observed 2–3 times more intragenomic variation for ITS2 than mt COI in both *S. dorsalis* and *T. palmi*.

Rugman *et al*. (2006) utilized internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of nuclear ribosomal DNA for molecular Identification of pest species of *Scirtothrips* (Thysanoptera: Thripidae).

Yeh *et al*. (2015) utilized the sequences of internal transcribed spacer 1 (ITS1) region of 15 agronomically important thrips, including several virus transmission species, in order to design species-specific primers for multiplex PCR and probes for microarray assay.

2.12. DNA sequencing

DNA sequencing is the process of determining the accurate order of the four nitrogen bases; adenine, thymine, guanine, cytosine in a specified DNA strand of organisms. The advent of DNA sequencing methods has emerged as an inevitable tool in various disciplines of science, ranging from archaeology, anthropology, genetics, biotechnology, molecular biology, forensic sciences (Franca *et al*., 2001).

DNA sequencing plays a vital role in medical diagnosis including cancer diagnostics and other viral diseases, to characterize antibody repertoire and can be used to guide patient treatment.

There are various methods to employ sequencing of DNA is available now. The first method was described by Sanger and Coulson was called ‘plus and minus’ method of DNA sequencing (Sanger & Coulson, 1975). The most recent one is Next-generation sequencing , a high throughput method which able to sequence millions of nucleotides in a single run. Next-generation sequencing can perform massively parallel way, it enables multiplexing many reaction and multiplex different specimens all on one run.

2.13. Next generation sequencing

Next-generation sequencing techniques cover a multitude of approaches depending on distinct technology. Though quite varied methods and biology were used in each phase from model library preparing, fragment amplification, to sequencing, they all embraced a huge matrix setup popularized by microarray assessment – DNA specimens on the grid are concurrently evaluated in conjunction. Though quite varied methods and biology were used in each phase from model library preparing, fragment amplification, to sequencing, they all embraced a huge matrix setup popularized by microarray assessment – DNA specimens on the grid are concurrently evaluated in conjunction (Zhou *et al.,* 2010). Based on the overall outline, several main features can be readily noted. First, huge parallelism can be accomplished by configuring an organized or disordered range that provides a large degree of density of information. Conceptually, this is restricted only by the diffraction limit of light (i.e., half of the wavelength used to detect autonomous optical occurrences. This drastically improves the general sequencing operation throughput. Secondly, no electrophoresis is used, leading in great ease of miniaturization and less sample / reagent usage over 1st-generation technology (Zhou *et al.,* 2010).

2.14. Phylogeny

Evolution can be described in terms of the characters that are changing in each population from the data regarding morphological characters, comparative embryology, fossil records and comparative anatomy to analyse the evolutionary similarities between organisms.

Phylogenetic methods plays an important role in estimating the evolutionary rates of genes and to detect footprints of natural selection (Yang *et al*., 2005).

Each leaf node in a phylogenetic tree represents species and each edge indicates the relationship status between neighbouring species and the length of an edge denotes the evolutionary distance between species (Choi *et al*., 2000).

For phylogenetic tree construction, several tools are available like NJPLOT, GENETREE, PHYLIP, GENEDOC, DAMBE, TREECON, TREEVIEW and SPECTRUM. Barrett *et al*. (2005) suggested using a 0.02 divergence as the threshold for species diagnosis with mtDNA-COI sequences.

Choi *et al*. (2000) developed a unified viewing tool *Phylodraw* which supports various multi-alignment formats like Dialign2, Clustal-W, Phylip format, Nexus, MEGA and pairwise distance matrix for phylogenetic tree construction.

2.15. Speciation and molecular divergence

Speciation in organisms occurred as a result of divergent natural selection. For example, adaptation to different ecological environments, via divergent selection, can result in the evolution of reproductive incompatibility between populations (Matsubayashi *et al*., 2010)

Walsh *et al*. (1867) proposed that insect speciation could be driven by shifting and adapting to new host plants.

Matsubayashi *et al*. (2010) has found that performance traits (those affecting growth and survival on different plants) in phytophagous insects occurs as a result of traits under selection, whereas preference traits (those involved in alighting, feeding, and oviposition preference) occurs as a result of reproductive isolation.

Parallel genetic divergence between insects can be affected by factors other than divergent selection, such as background selection and reduced recombination (Barrett *et al*., 2010).

The branching orders in phylogenies can be inferred directly from distributions of qualitative character states, using cladistics, parsimony or maximum likelihood analyses, which are valid irrespective of whether molecules evolve in strictly time-dependent fashion.

3. MATERIALS AND METHODS

The study on "DNA barcoding of thrips (Thysanoptera: Thripidae) on selected vegetable crops of Kerala" was done at Regional Agricultural Research Station, Pattambi and Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University during the period 2019-2020. The materials used and methodologies adopted are presented in this chapter.

3.1. Purposive survey

Thrips infested leaf samples were collected from diverse localities of Thrissur and Palakkad districts of Kerala from the vegetable crops; cucumber, coccinia, chilli, cowpea, brinjal, tomato separately in polythene bags from each locality and brought to the laboratory. From the laboratory, leaves were clearly observed under stereo-microscope and photographs of specimens were taken.

3.2. Storage of specimens

Specimens from each selected vegetable crops were stored separately in 70% and 100% alcohol in 2ml vials. Thrips stored in 70% ethanol at room temperature was identified Zoological Survey of India, Kolkata. Thrips stored in 100% ethanol at -20 ^{0} C were used for DNA extraction.

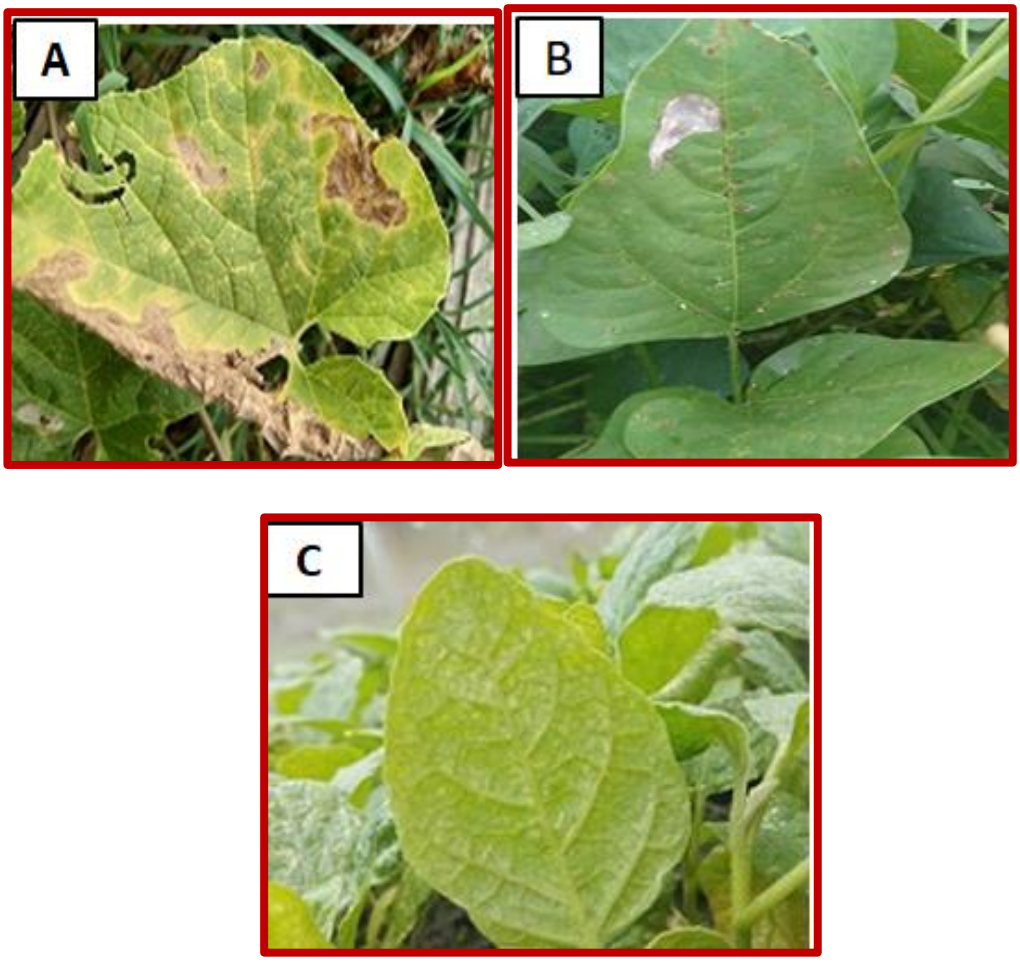


Plate 1. Thrips infected leaves collected from the vegetable crops; A- Cucumber, B- Cowpea, C- Brinjal

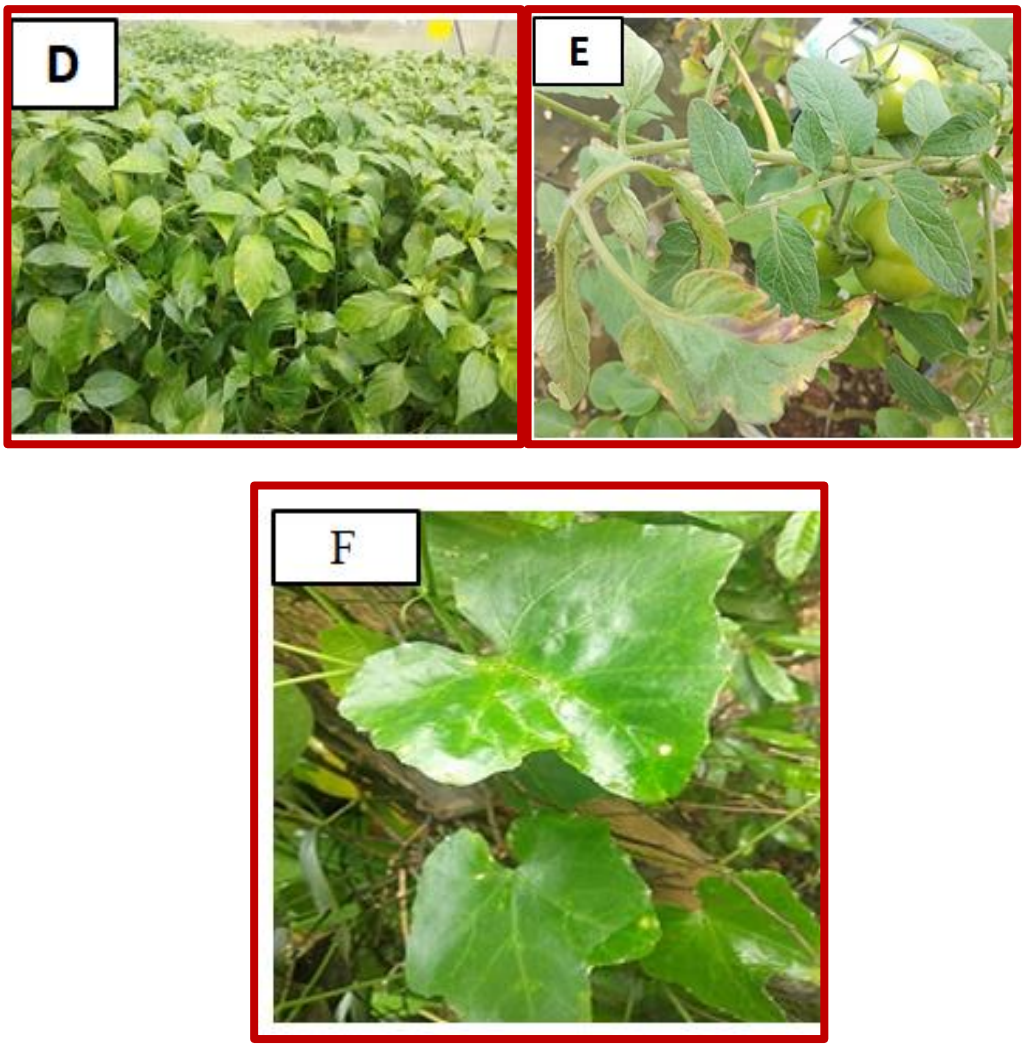


Plate 2. Thrips infected leaves collected the vegetables crops D-Chilli, E- Tomato, F-Coccinia

Plate 3. Survey done at various locations of Thrissur and Palakkad districts; A- Hightech horticulture (Thrissur), B- Chalissery (Palakkad), C- Peringode (Palakkad), D- Kattakampal (Thrissur)

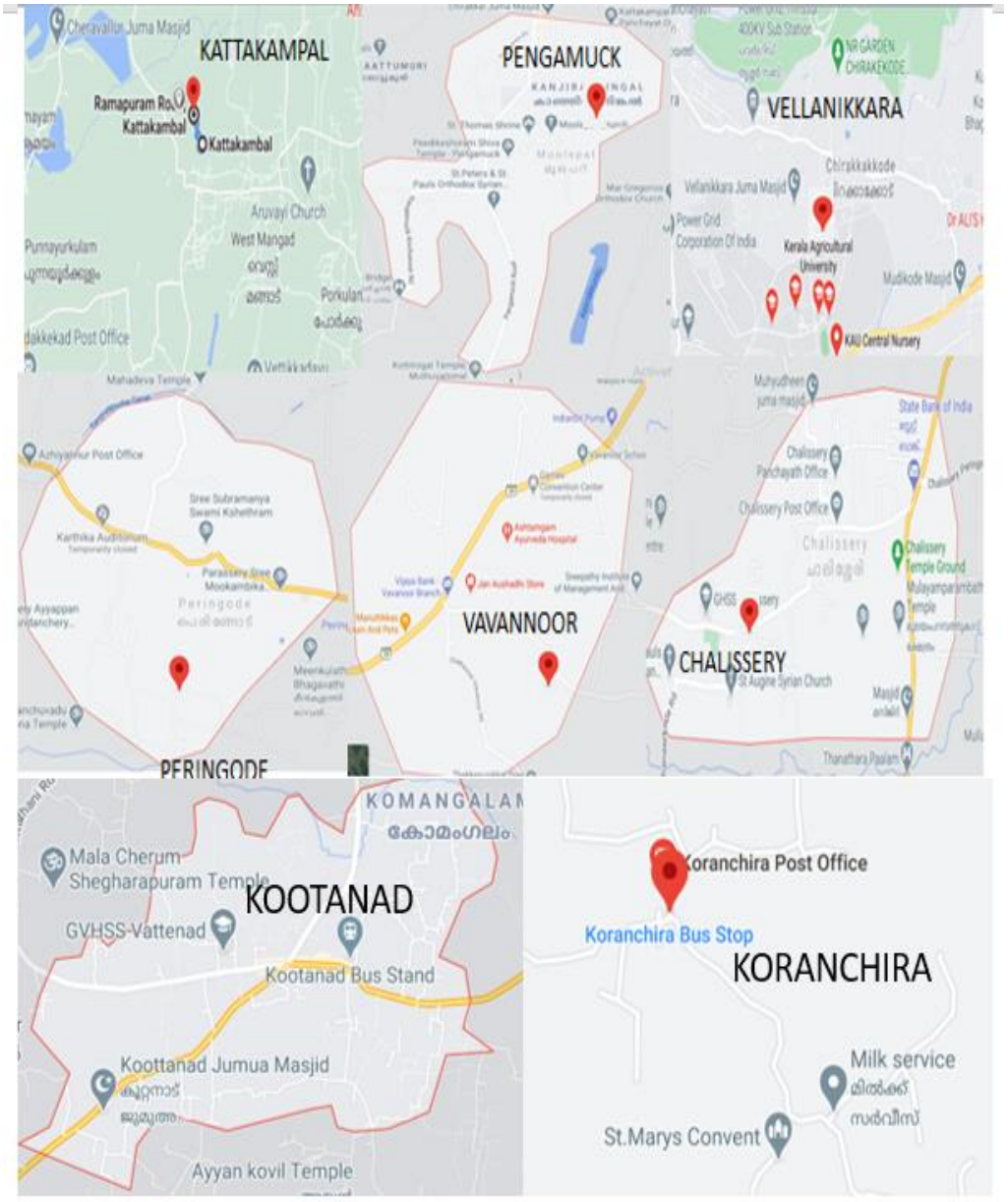


Plate 4. Locations covered for sample collection

Plate 5. Materials used for observation and storage of thrips samples.

3.3. Identification of thrips specimens

Morphological characters of different thrips species were identified by experts in thrips taxonomy using particular taxonomic keys.

Taxonomic keys to identify *Thrips florum* (Schmutz *et al.*, 1913)

- Brownish in color.
- Dark yellow coloured tibiae with light brown coloured femora
- 7 segmented antennae (seldom 8)
- Antennal segment III with yellow colour. Antennae will be 7 or 8 segmented; ocellar setae III arise outside ocellar triangle; postocular setae I and III comparatively larger than postacular seta II.
- Pronotum is transverse, weak midlateral setae.
- Apodeme will be posterior and submarginal.

- Longitudinal metanotum transversely striate on anterior half, with broadly spaced striations on posterior half, median setae at anterior margin, campaniform sensilla also present.
- Forewings will be brownish and underneath will be pale in colour. Forewings first vein with 3 setae on distal half, terminal seta is much smaller than clavus with subterminal seta.
- Four lateral setae containing abdominal tergite II , short and irregular tergite VIII comb, sternites III–VII with 6–14 distal setae.
- Males will be light brown in color.

Taxonomic keys to identify *Thrips palmi* (Karny, 1925)

- Yellowish body and legs with light brown coloured major setae
- Distal antennal segments IV & V with brown colour
- Wider head, small ocellar setae pair III and erects out outocellar triangle; postocular setae pair I slightly longer than ocellar setae III.
- Antennae will be Seven segmented with III & IV slightly constricted at apex and VII will be very short.
- Pronotum with 2 pairs of long postero-angular setae, Three pairs of setae present in posterior margin. Metanotum with irregular longitudinal lines converging to posterior margin, median setae arouse behind anterior margin
- Campaniform sensilla present.
- Forewings will be pale in color.
- Forewing first vein with 3 (or 2) setae on distal half and second vein with row of about 15 setae. Tergite II containing 4 lateral marginal setae. Posterior margin of tergite VIII with complete comb of long slender microtrichia, pleurotergites without distal setae.
- Two pairs of marginal setae on sternite II and III–VII with 3 pairs, median pair on VII erects in front of margin.
- Females occur much larger than males; tergite VIII with marginal comb present medially.

Taxonomic keys to identify *Thrips hawaiiensis* (Morgan, 1913)

- Brownish body and also seen as bicolored with brown abdomen.
- Head and thorax is orange yellow coloured .
- Antennae 7- or 8-segmented with yellowish Antennal segment III.
- Ocellar setae III projects out ocellar triangle, subequal postocular setae I & II.
- Transversely marked pronotum with one pair of midlateral setae, weak posterior sub-marginal apodeme. Mesonotum with lines of sculpture close to anterior campaniform sensilla.
- Metanotum transversely striate on anterior half, with longitudinal but more broadly spaced striations on posterior half, median setae projects at anterior margin, campaniform sensilla present.
- Forewings are brown and pale underneath.
- Forewing first vein with 3 setae on distal half, terminal seta are much longer than clavus
- Four lateral setae containing abdominal tergite II; short and irregular tergite VIII comb; 12–25 discal setae present in sternites III–VII.
- Male usually pale brown in color.

Taxonomic keys to identify *Ayyaria chaetophora* (Wilson, 1975)

- Dark brown coloured body, yellowish mid and hind tibiae
- Slender segmented antennae with segment III–IV with forked sensorium.
- Head slightly wider in appearance, with three pairs of ocellar setae in which pair III longer than the side of ocellar triangle, arising between anterior margins of hind ocelli.
- Pronotum with one pair of long anteromarginal setae, and two pairs of long posteroangular setae; pronotal surface with little sculpture.
- Metanotum present without sculpture medially
- White forewings with two dark cross bands. Forewing first vein with three setae on distal half and second vein contains only three setae.

- Males almost similar to female but smaller in appearance; tergite IX with two stout thorn-like setae each present on large tubercle, posterior to these have five small tubercles.

Table 3.1. Description of the samples collected

SL.NO	ACCESSION NO	CROP	PLACE	DISTRICT	IDENTIFIED SPECIES
1.	VKCU1	Cucumber	Vellanikkara	Thrissur	*Ayyaria chaetophora*
2.	VVCU2	Cucumber	Vavanoor	Palakkad	*Ayyaria chaetophora*
3.	CYCU3	Cucumber	Challissery	Palakkad	*Ayyaria chaetophora*
4.	IFCI4	Chilli	Instructional Farm	Thrissur	*Thrips florum*
5.	VKCI3	Chilli	Vellanikkara	Thrissur	*Thrips florum*
6.	PNCI2	Chilli	Peringode	Palakkad	*Thrips florum*
7.	CYCI1	Chilli	Challissery	Palakkad	*Thrips florum*
8.	KKCP1	Cowpea	Kattakampal	Thrissur	*Ayyaria chaetophora*
9.	PMCP2	Cowpea	Pengamuck	Thrissur	*Ayyaria chaetophora*
10.	CYCP4	Cowpea	Challissery	Palakkad	*Ayyaria chaetophora*
11.	VVCP3	Cowpea	Vavanoor	Palakkad	*Ayyaria chaetophora*
12.	KKCC1	Coccinia	Kattakampal	Thrissur	*Ayyaria chaetophora*
13.	KRCC2	Coccinia	Koranchira	Palakkad	*Thrips hawaiienesis*
14.	PNCC3	Coccinia	Peringode	Palakkad	*Ayyaria chaetophora*
15.	HHTO1	Tomato	Hitech horticulture	Thrissur	*Thrips palmi*
16.	CYTO2	Tomato	Challissery	Palakkad	*Thrips palmi*
17.	VVTO3	Tomato	Vavanoor	Palakkad	*Thrips palmi*

18.	HHBR1	Brinjal	Hitech horticulture	Thrissur	*Thrips palmi*
19.	VVBR3	Brinjal	Vavanoor	Palakkad	*Thrips palmi*
20.	KKBR3	Brinjal	Kattakampal	Thrissur	*Ayyaria chaetophora*
21.	KUBR2	Brinjal	Kootanad	Palakkad	*Ayyaria chaetophora*

3.5. DNA isolation

DNA isolation of thrips specimens was done using Qiagen DNeasy blood and tissue kit by following procedure.

Procedure:

1. Three to four thrips specimens were taken in autoclaved 1.5ml eppendorf tube containing 180µl buffer ATL and crushed using micro pestle. Then 20µl proteinase k also added to the eppendorf tube to prevent protein contamination.
2. The samples were vortexed for 20s and incubated in waterbath at 65 ^{0}C for complete lysis which took 3- 4 hours.
3. Vortexed again for 20s. Then 200µl Buffer AL added to the sample, and mixed thoroughly by vortexing. After that 200µl ethanol (96–100%) was added, and again vortexed thoroughly.
4. Pipeted the mixture into the DNeasy Mini spin column placed in a 2ml collection tube.
5. Centrifuged at ≥6000x g (8000rpm) for 1min and discarded the collection tube.
6. 500µl Buffer AW1 was added to the DNeasy Mini spin column in a new 2ml collection tube to ensure that mini spin column is free from ethanol and again centrifuged for 1min at ≥6000x g (8000rpm) and discarded the flow-through and collection tube.

7. 500µl Buffer AW2 was added to the DNeasy Mini spin column in a new 2ml collection tube and centrifuged for 3min at 20,000x g (14,000rpm) to avoid moisture in the DNeasy membrane and discarded the flow-through and collection tube.
8. 200µl of Buffer AE added directly onto the DNeasy membrane which was kept in a clean 2ml microcentrifuge tube.
9. Incubated at room temperature for 1min, and then centrifuged for 1min at ≥6000x g (8000 rpm) to elute the DNA.

3.6. Quality Assessment of DNA

The most common technique to determine DNA yield and purity is the measurement of absorbance by using UV- Visible spectrophotometer. DNA absorbs light actively at 260 nm whereas proteins shows absorbance maxima at 280nm. Absorbance of each samples recorded at both wavelengths and the purity of samples were indicated by the ratio OD260/OD280. The absorbance value of 1.8 states that the DNA is pure and free from proteins and RNA. DNA quantity can be assessed in a sample by utilizing the equation.

1 OD at 260nm = 50µg DNA/µl

Therefore, OD260 X 50 gives the quantity of DNA in µg/µl

3.7. PCR amplification

The polymerase chain reaction (PCR) functions only by providing adequate conditions involving proper proportions of the components in the reaction mixture including DNA template, Taq assay buffer A, Taq DNA polymerase, dNTPs , forward and reverse primers. Aliquot of this master mix were discharged into 0.2 ml PCR tubes and subjected to thermal cycling.

Table 3.2. Components of PCR reaction mix

Sl. No	**Components**	**Quantity in µl**
1	Sterile distilled water	33.5
2	10X Taq assay buffer A	6.00
3	dNTP mix (10Mm each)	3.00
4	3U Taq DNA polymerase	0.50
5	Forward primer (10Mm)	1.00
6	Reverse primer (10Mm)	1.00
7	Genomic DNA	5.00
Total		50.00

Table 3.3. PCR program

Step	**Temperature (^{0C})**	**Duration**
Initial Denaturation	95 ^{0}C	3 minutes
Denaturation	95 ^{0}C	1 minute
Annealing	50 ^{0}C	1 minute 30 seconds
Extension	72 ^{0}C	1 minute 30 seconds
Final Extension	72^{0}C	10 minutes (35 cycles)

Table 3.4. Details of primers used for DNA barcoding

Sl.No	Locus	Sequence	Annealing Temperature (^{0}C)	Product length (bp)	Reference
1	COI (F)	GGAGGATTTGGAAATTGATTAGTTCC	50	890	Simon et al., 1994
2	COI (R)	GATAAAACGTAATGAAAATGAGCTAC			

3.8. Agarose gel electrophoresis (AGE)

Agarose gel electrophoresis regarded as the most efficient method to separate DNA fragments of varying size range. Amplification of CO1 locus was detected through agarose gel electrophoresis on 2% agarose gel.

Procedure for Agarose gel electrophoresis

1. Initial step for gel casting was to make the electrophoresis gel tray available for carrying the gel. Both ends of the tray correctly sealed with tape. Above the tray comb was placed vertically in such a way that comb teeth were dipped about 1 to 2mm to the tray surface.
2. Agarose (2%) were prepared in a 200 ml glass conical flask by adding 1.2g agarose in 80ml 1X TAE buffer (1.2ml TAE from stock made up to 100ml). For proper dissolving of agarose, heat was provided for 50 to 65 seconds using a microwave oven.
3. Heated agarose solution was brought to room temperature. After attaining room temperature very small quantity EtBr (2 µl) was added to the agarose solution.
4. Agarose gel solution was poured carefully into the gel tray by filling the teeth of comb initially placed.

5. Proper solidification of gel was made sure. Then slowly the already placed comb and tape for tightening the gel tray were removed carefully. The

   electrophoresis tray with agarose gel was placed in electrophoresis chamber, and filled with 1X electrophoresis buffer.
6. The molecular weight ladder of 100 bp was loaded to one of the well. Then samples for carrying out gel electrophoresis were made by adding 2µl of 6X gel loading dye for each 8 µl of DNA and allow proper mixing by pipetting. Each DNA sample were loaded as 10 µl to all wells.
7. Electrophoresis process was done at 70 volts until the dye has migrated to two third of the length of the gel. The time took for this process was nearly 3 hours. With the help of Gel doc, the sample was documented

3.9. Sequencing of PCR amplified products

DNA extracted from 21 thrips specimens were amplified with the COI primer. These PCR amplified products of thrips were 890 bp which was sequenced at AgriGenome, Ernakulam for sequencing.

3.10. Data Analysis

3.10.1. Sequence Annotation

Presence of internal stop codons within the COI sequences were investigated using MEGA 7 software by aligning the whole sequences in the software. COI sequences containing internal stop codons were removed by using BioEdit software to make the sequences free from stop codons.

3.10.2. Sequence homology analysis

By using the sequence similarity checking tool Basic Local Alignment Search Tool (BLASTn) of NCBI, query sequence was annotated with a database to identify the homology sequences.

3.10.3. Barcode gap analysis

Barcode gap can be defined as the difference between inter- and intraspecific genetic distances within species and between species of an organism. Barcode Gap Analysis method could help to anticipate the distribution of distances within each species and the distance to the nearest neighbour of each species. Sequences were readily aligned using clustalW tool of MEGA7 software to identify the barcode gaps.

3.10.4. Phylogenetic Analysis

Phylogenetic analysis of COI sequences of thrips species was carried out by using MEGA7 software, which could discriminate all the species at phylogeny level. The sequences were aligned using clustalW tool of MEGA7 software and the phylogenetic tree was created by using 'maximum parsimony model' from 'phylogeny' tool of MEGA7 software.

3.10.5. Pair wise distance calculation

Evolutionary distances between thrips species were calculated using Kimura 2 parameter from MEGA7 software. From the 'Distance' tool, "Compute Pair-wise" command were used to create the pairwise distance of species.

3.10.6. Submission to Barcode of Life Data System

Information regarding thrips specimens including specimen data, specimen images, sequence information and trace files were deposited into Barcode of Life Data System (BOLD), an online molecular platform for researchers and students to unveil the barcode data and to contribute novel barcodes of organisms (http://www.boldsystems.org).

3.10.7. Submission to GenBank (NCBI)

A dataset of 21 thrips species with sequences was submitted to NCBI GenBank. Sequence submission was carried out through 'Submission Portal' of NCBI.

The procedure for sequence submission is as follows:

(i) Initially NCBI account for sequence submission were created with strong

(ii) password. Then logged in to the account in MyNCBI at http://www.ncbi.nlm.nih.gov/guide/howto/submit-sequence-data/ website.

(iii) All sequences in FASTA format and information like date for public release (immediate or at a specified future date), basic information (authors and a working title) for a corresponding reference paper, name of the organism or plant from which the sequence data were isolated and collection details were also entered.

(iv) The accession numbers to all the submitted sequences were generated.

4. RESULTS

The study entitled 'DNA barcoding of thrips (Thysanoptera: Thripidae) in selected vegetable crops of Kerala' has been conducted at Regional Agricultural Research Station, Pattambi and Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University. The primary objective of the study was to generate DNA barcodes for different species of thrips infesting selected vegetable crops of Kerala and to find out the variability among them. The results of the current study are described in this chapter.

4.1. Purposive survey

Thrips infected leaf samples of the selected six vegetable crops; chilli, cowpea, cucumber, coccinia, brinjal, tomato were collected from different locations of Thrissur and Palakkad districts. Thrips species are mostly seen in warm climatic conditions. So samples were collected mostly in afternoon. Thrips specimens from each selected vegetable crops were stored separately in 70% and 100% alcohol in 2ml vials. The freshly collected thrips specimens were instantly transferred to 70% ethanol and 100% ethanol in 2 ml vials and were stored in room temperature and -20^0 C refrigerator respectively. Specimens stored in 70% ethanol at room temperature have been sent to Zoological Survey of India, Kolkata for identification purposes and thrips stored in 100% ethanol at -20^0 C were used for DNA extraction. Table shows the details of specimens collected. Images of the specimens are shown in plates.

Table 4.1. Details of samples

Sl. No	Accession No	Crop	Place	District	Identified species
1.	VKCU1	Cucumber	Vellanikkara	Thrissur	*Ayyaria chaetophora*
2.	VVCU2	Cucumber	Vavanoor	Palakkad	*Ayyaria chaetophora*
3.	CYCU3	Cucumber	Challissery	Palakkad	*Ayyaria chaetophora*
4.	IFCI4	Chilli	Instructional Farm	Thrissur	*Thrips florum*
5.	VKCI3	Chilli	Vellanikkara	Thrissur	*Thrips florum*
6.	PNCI2	Chilli	Peringode	Palakkad	*Thrips florum*
	CYCI1	Chilli	Challissery	Palakkad	*Thrips florum*
7.	KKCP1	Cowpea	Kattakampal	Thrissur	*Ayyaria chaetophora*
8.	PMCP2	Cowpea	Pengamuck	Thrissur	*Ayyaria chaetophora*
9.	CYCP4	Cowpea	Challissery	Palakkad	*Ayyaria chaetophora*
10.	VVCP3	Cowpea	Vavanoor	Palakkad	*Ayyaria chaetophora*
11.	KKCC1	Coccinia	Kattakampal	Thrissur	*Ayyaria chaetophora*
12.	KRCC2	Coccinia	Koranchira	Palakkad	*Thrips hawaiienesis*
13.	PNCC3	Coccinia	Peringode	Palakkad	*Ayyaria chaetophora*
14.	HHTO1	Tomato	Hitech horticulture	Thrissur	*Thrips palmi*
15.	CYTO2	Tomato	Challissery	Palakkad	*Thrips palmi*
16.	VVTO3	Tomato	Vavanoor	Palakkad	*Thrips palmi*
17.	HHBR1	Brinjal	Hitech horticulture	Thrissur	*Thrips palmi*

18.	VVBR3	Brinjal	Vavanoor	Palakkad	*Thrips palmi*
19.	KKBR3	Brinjal	Kattakampal	Thrissur	*Ayyaria chaetophora*
20.	KUBR2	Brinjal	Kootanad	Palakkad	*Ayyaria chaetophora*

Thrips hawaiiensis

Thrips florum

Ayyaria chaetophora

Thrips palmi

Plate 1. Images of specimen collected

4.2. Molecular analysis

4.2.1. DNA isolation

DNA isolation of thrips specimens were conducted by using Qiagen DNeasy blood and tissue kit by following the kit procedure. The agarose gel electrophoresis has indicated clear bands without contamination and spectrophotometric analysis gave a ratio of 1.8 indicated good quality DNA.

4.2.2. Quality assessment of DNA

The purity of isolated DNA has been checked using spectrophotometer. Nucleic acids shows absorption maxima at 260 nm whereas proteins shows absorbance maxima at 280 nm. Absorbance of each samples recorded at both wavelengths and the purity of samples was indicated by the ratio OD260/OD280. The absorbance value of 1.8 specifies that the DNA is pure and free from proteins and RNA. Spectrophotometric analysis gave ratio of UV absorbance ratio (A260/280) around 1.8. Quality and quantity of DNA isolated from thrips species assessed by spectrophotometer method are represented in the table below.

Table 2. Quality and quantity of DNA isolated from Thrips species assessed by spectrophotometeric method

Sample ID	**UV absorbance at 260 nm (A260)**	**UV absorbance at 280 nm (A280)**	**A260/A280**	**Concentration (µg/µl)**
Ifci4	2.08	1.13	1.84	105.4
Kkbr3	2.34	1.21	1.93	118
Kkcc1	1.99	0.99	2.01	98
Pnci2	2.46	2.34	1.83	112.02

Pmcp2	1.04	0.54	1.86	109.9
Vkci3	0.65	0.35	1.86	45
Cyci1	0.98	0.45	2.18	38
Kubr2	2.93	1.54	1.90	112.31
Hhbr1	2.91	1.61	1.82	118.92
Vvbr3	2.54	1.33	1.91	119.27
Kkcp1	2.78	1.49	1.87	111.53
Vvcp3	2.09	1.08	1.93	105.01
Cycp4	1.04	0.56	1.86	92.56
Krcc2	2.44	1.21	2.02	114.43
Pncc3	0.87	0.46	1.89	84.75
Vkcu1	1.95	0.98	1.99	48.44
Vvcu2	1.63	0.89	1.83	92.04
Cycu3	2.74	1.48	1.85	118.88
Hhto1	1.93	1.05	1.84	93.22
Cyto2	2.38	1.26	1.89	104.53
Vvto3	2.91	1.52	1.91	122.02

4.2.3. PCR Amplification of CO1 loci

The region of mtDNA containing cytochrome oxidase I (COI), has been widely used in systematic and population genetic studies of insect including thrips. The CO1 marker from mitochondrial cytochrome c Oxidase subunit I gene, were used for generating species specific signature sequences from mitochondrial genome. The DNA was amplified by using PCR amplification of CO1 loci. The PCR assay was standardized by increasing the annealing temperature and time which made adequate results.

4.2.4. Confirmation of PCR amplification

PCR amplification of the candidate locus was then confirmed through 2 per cent agarose gel electrophoresis. The DNA barcoding primers amplified at the expected product size of 890 bp.

4.2.5. Sequencing of PCR products

The confirmed PCR products of COI primers by agarose gel electrophoresis were sequenced with specific set of primers. The sequencing was done at AgriGenome Lab. Pvt. Ltd., Cochin.

Amplification of COI (890 bp) regions of thrips using the barcoding primers

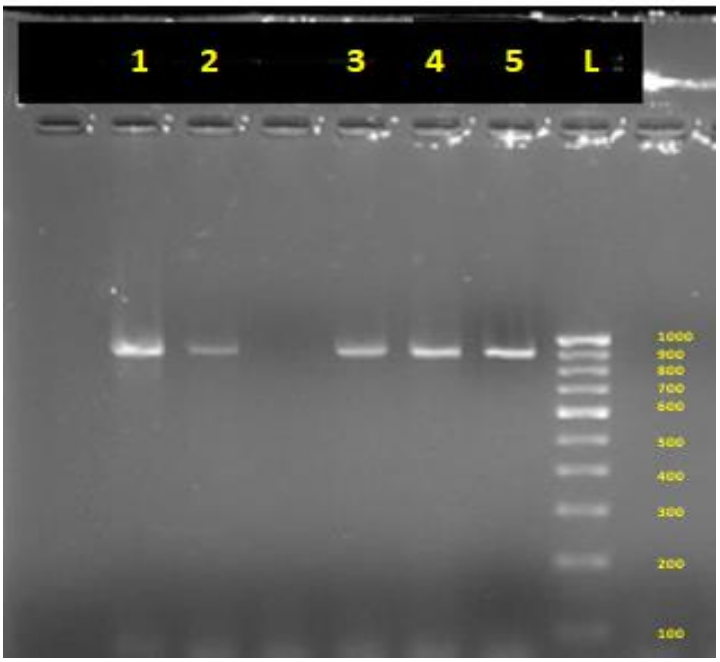


Fig 1: 1-ifci4, 2-kkbr3, 3-kkcc1, 4-pnci2, 5-pmcp2, L-ladder

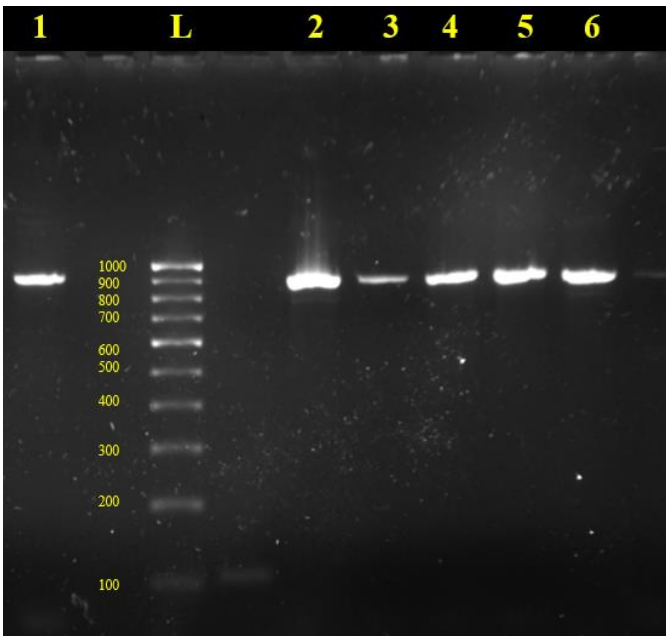


Fig 2: 6-vkci3, L-Ladder, 7-cyci1, 8-kubr2, 9-hhbr1, 10-vvbr3, 11-kkcp1

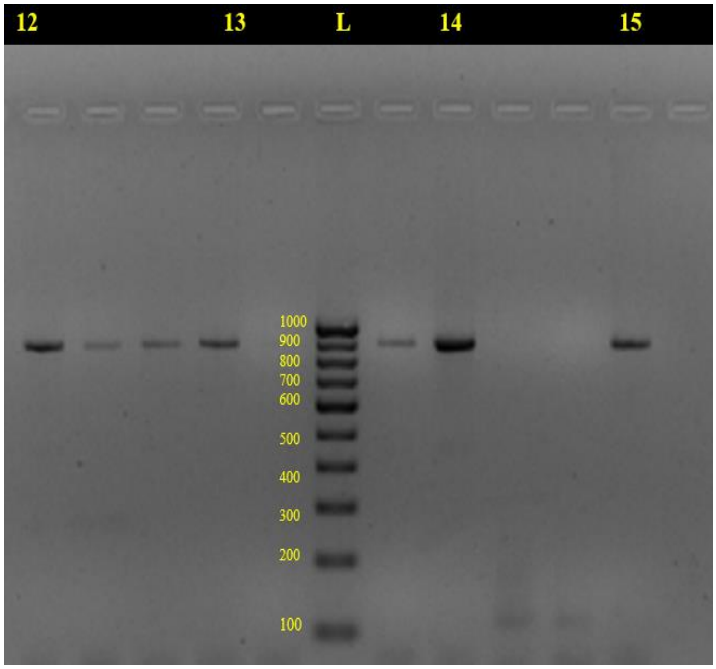


Fig 3: 12-vvcp3, 13-cycp4, L-Ladder 14-krcc2, 15-pncc3

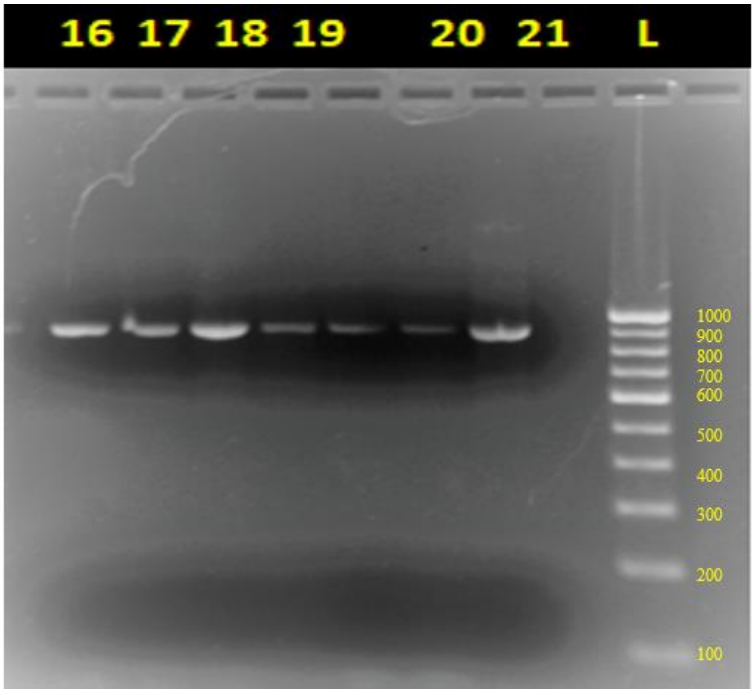


Fig 4: 16-vkcu1, 17-vvcu2, 18-cycu3, 19-hhto1, 20-cyto2, 21-vvto3

4.5. Data analysis

4.5.1. Analysis of sequence homology using BLASTn tool

The homology of 21 sequences generated from 21 thrips specimen were analysed with the nucleotide database using BLASTn tool. The results obtained is presented in figure 9.

4.5.2. Sequence annotation

CO1 sequences containing internal stop codons were removed using BioEdit software to make the sequences free from stop codons.

4.5.3. Barcode gap analysis

Barcode Gap Analysis predict the distribution of distances within each species and the distance to the nearest neighbour of each species. Sequences were readily aligned using clustalW tool of MEGA7 software to identify the similarity in nitrogen bases of the sequences. Multiple sequence alignment revealed highest similarity between *Thrips florum* and *Thrips hawaiiensis* and showed base difference only in the position of guanine in *T. hawaiiensis* in case of adenine in *T. florum*. Barcode gap analysis has shown the presence of cytosine in *T.palmi* but thymine in *Ayyaria chaetophora*.

4.5.4. Phylogenetic analysis

Phylogenetic tree constructed by using MEGA7 software (Maximum parsimony model) for COI sequences of thrips species are shown in figure 6. These results specified that *T. hawaiienesis* and *T. florum* are closely related species.

4.5.5. Pairwise distance calculation

Inter specific distance between species were computed using K2P (Kimura 2 parameter) from MEGA7 software. The “Distance|Compute Pair-wise” command created the pair wise distances. The results of pairwise distance of thrips is presented in figure 7.

In the present study, the range of distance within *T. florum* was 0% to 0.005%. Distance between *T. florum* and *T. hawaiiensis* were 0.003% to 0.005%. Distance between *Ayyaria chaetophora* and *T.palmi* were 0.259% to 0.267%.

4.5.6. Submission to Barcode of Life Data System (BOLD)

Data regarding the specimen taxonomy, specimen images, sequence information and trace files were deposited into BOLD database. Specific process IDs for each specimen were generated by BOLD and subsequently barcodes were also generated. The list of process IDs generated by BOLD are given in table 5. Details of BOLD (Barcodes of Life Data) submission with data including taxonomy of specimens and the illustrated barcodes, nucleotide sequences and aminoacid sequences is shown in figure 9.

4.5.7. Submission to NCBI

DNA sequences of 21 thrips species were deposited in the NCBI GenBank database and the list of accession numbers generated for each sequence are given in table 5.

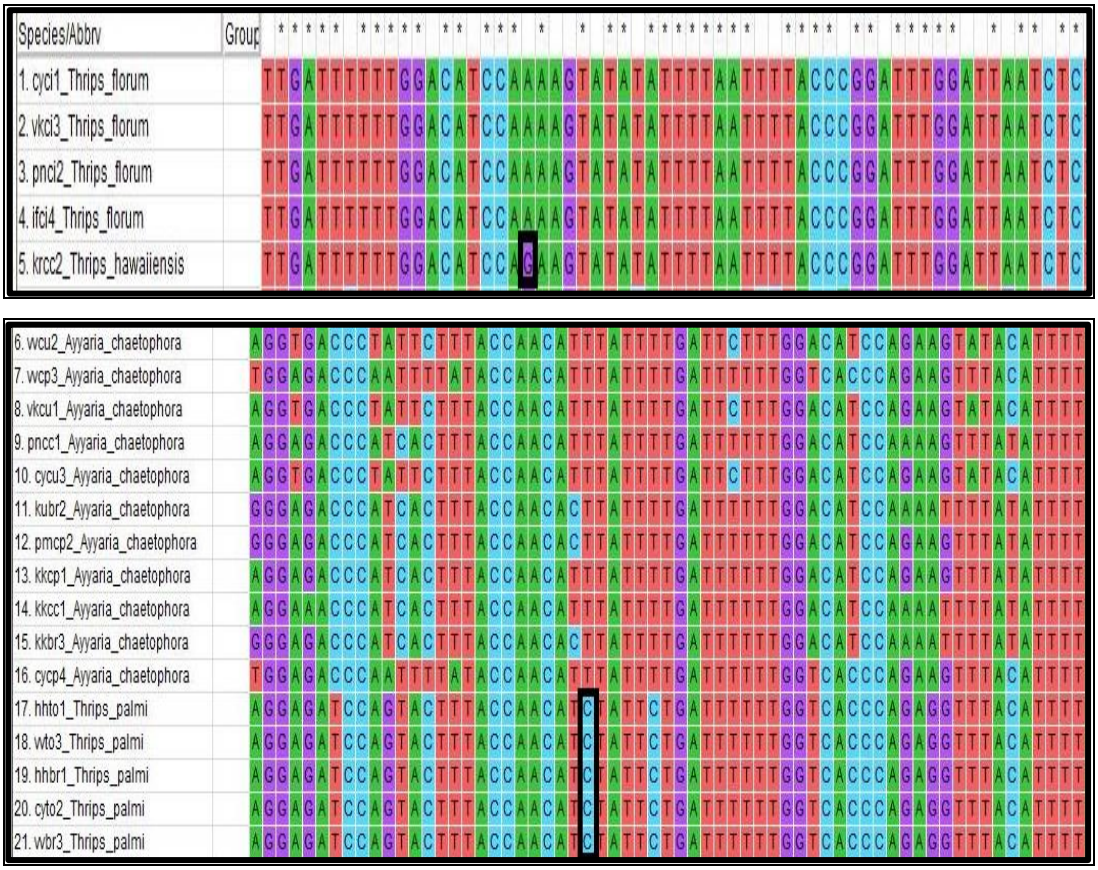


Fig 5: Barcode gap analysis of COI sequences of thrips species

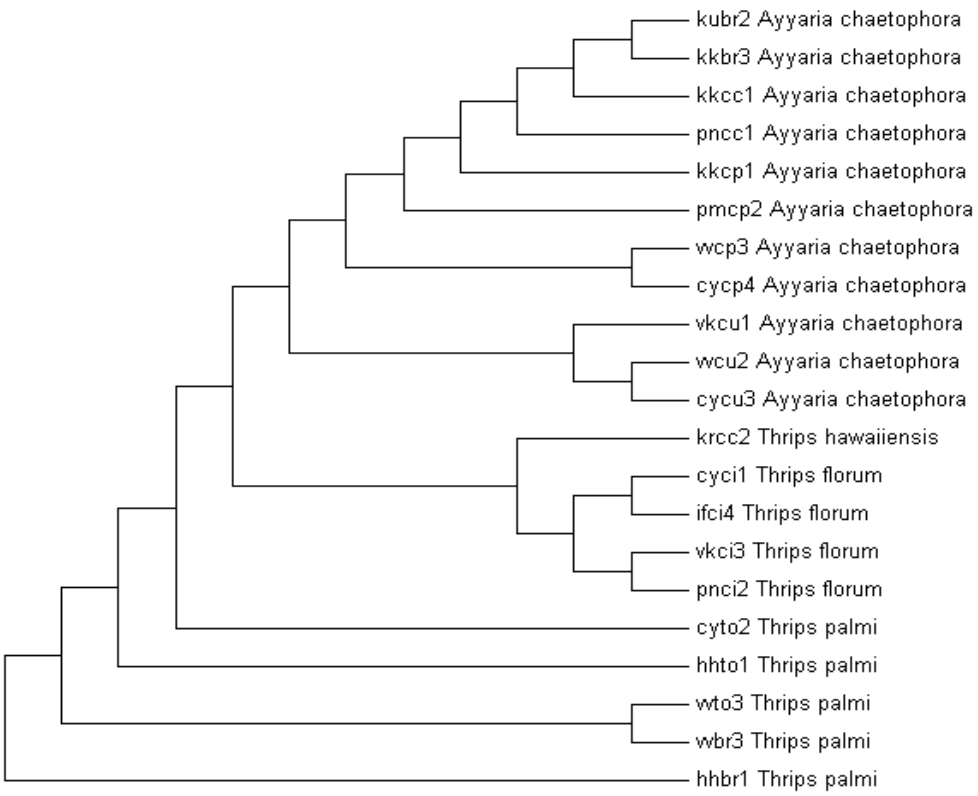


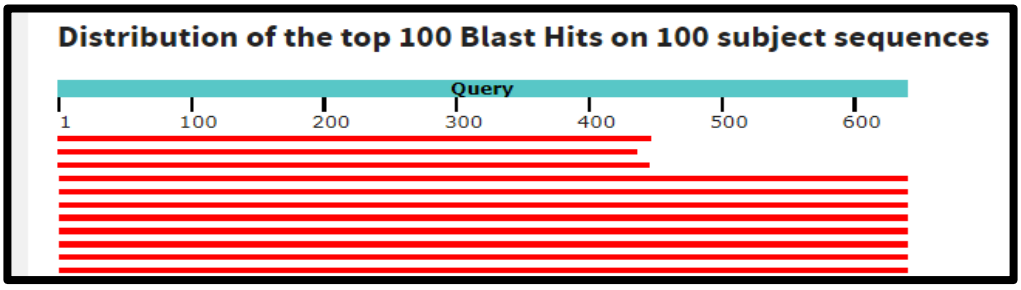
Fig 6: Phylogenetic tree drawn using MEGA7 software for COI sequences of thrips species.

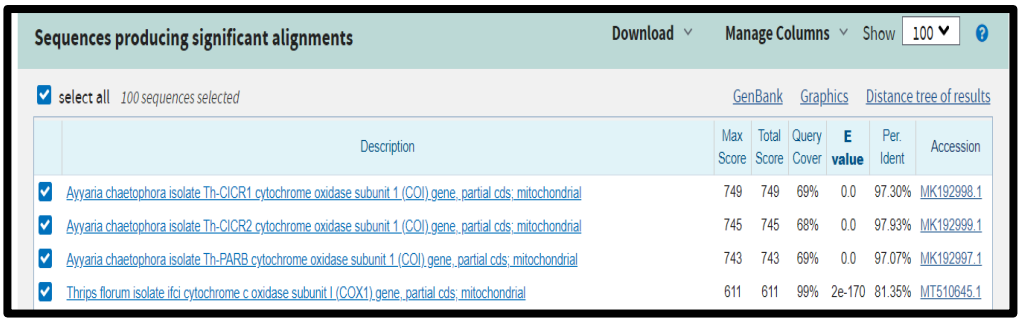
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. cyci1 Thrips florum																					
2. vkci3 Thrips florum	0.005																				
3. pnci2 Thrips florum	0.005	-0.000																			
4. ifci4 Thrips florum	-0.000	0.005	0.005																		
5. krcc2 Thrips hawaiiensis	0.003	0.005	0.005	0.003																	
6. vvcu2 Ayyaria chaetophora	0.224	0.226	0.226	0.224	0.219																
7. vvcp3 Ayyaria chaetophora	0.283	0.286	0.286	0.283	0.278	0.278															
8. vkcu1 Ayyaria chaetophora	0.224	0.226	0.226	0.224	0.219	-0.000	0.278														
9. pncc1 Ayyaria chaetophora	0.214	0.216	0.216	0.214	0.214	0.283	0.238	0.283													
10. cycu3 Ayyaria chaetophora	0.224	0.226	0.226	0.224	0.219	-0.000	0.278	-0.000	0.283												
11. kubr2 Ayyaria chaetophora	0.232	0.230	0.230	0.232	0.232	0.306	0.262	0.306	0.025	0.306											
12. pmcp2 Ayyaria chaetophora	0.223	0.225	0.225	0.223	0.218	0.290	0.245	0.290	0.018	0.290	0.034										
13. kkcp1 Ayyaria chaetophora	0.216	0.218	0.218	0.216	0.212	0.281	0.236	0.281	0.002	0.281	0.027	0.017									
14. kkcc1 Ayyaria chaetophora	0.225	0.228	0.228	0.225	0.225	0.293	0.250	0.293	0.010	0.293	0.022	0.029	0.012								
15. kkbr3 Ayyaria chaetophora	0.232	0.230	0.230	0.232	0.232	0.306	0.262	0.306	0.025	0.306	-0.000	0.034	0.027	0.022							
16. cycp4 Ayyaria chaetophora	0.283	0.286	0.286	0.283	0.278	0.278	-0.000	0.278	0.238	0.278	0.262	0.245	0.236	0.250	0.262						
17. hhto1 Thrips palmi	0.180	0.182	0.182	0.180	0.175	0.234	0.259	0.234	0.243	0.234	0.267	0.252	0.241	0.255	0.267	0.259					
18. vvto3 Thrips palmi	0.180	0.182	0.182	0.180	0.175	0.234	0.259	0.234	0.243	0.234	0.267	0.252	0.241	0.255	0.267	0.259	-0.000				
19. hhbr1 Thrips palmi	0.180	0.182	0.182	0.180	0.175	0.234	0.259	0.234	0.243	0.234	0.267	0.252	0.241	0.255	0.267	0.259	-0.000	-0.000			
20. cyto2 Thrips palmi	0.180	0.182	0.182	0.180	0.175	0.234	0.259	0.234	0.243	0.234	0.267	0.252	0.241	0.255	0.267	0.259	-0.000	-0.000	-0.000		
21. vvbr3 Thrips palmi	0.180	0.182	0.182	0.180	0.175	0.234	0.259	0.234	0.243	0.234	0.267	0.252	0.241	0.255	0.267	0.259	-0.000	-0.000	-0.000	-0.000	

Fig 7: Pairwise distance of COI sequences of thrips species

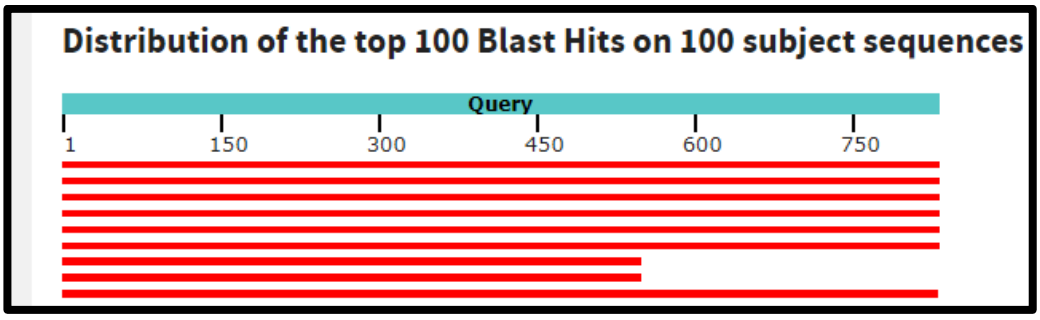
Fig 8: BLASTn results of selected sequences

Sample ID: vkcu1 (*Ayyaria chaetophora)*

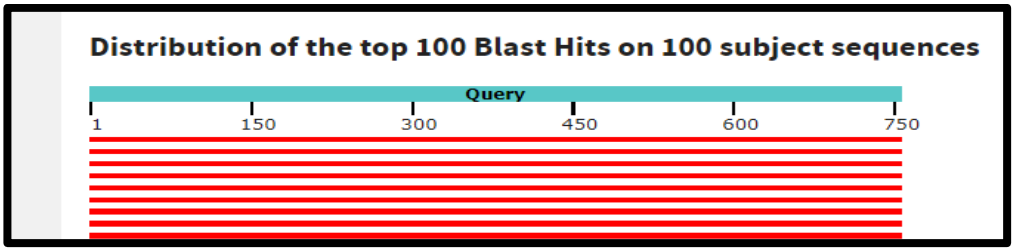




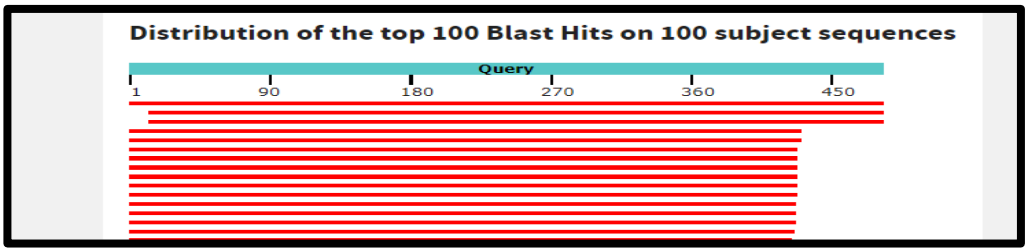
Sample ID: hhto1 (*Thrips palmi*)



Sample ID: cyci1 (*Thrips florum)*



Sample ID: krcc2 (*Thrips hawaiiensis*)



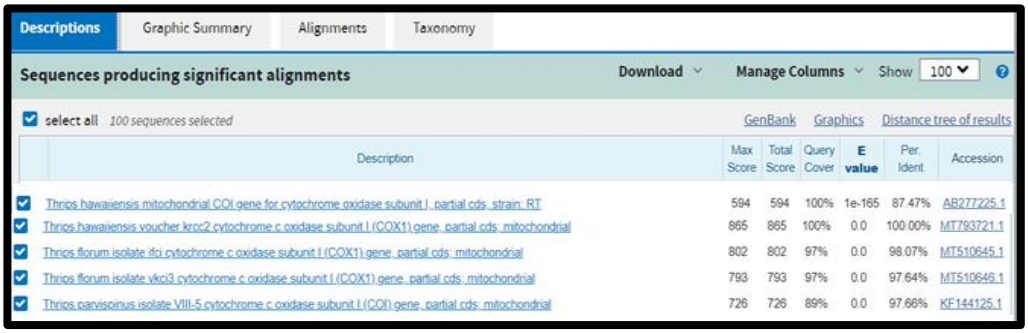


Fig 9: BOLD submissions

krcc2 - DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala [TDB]

krcc2

Namitha TH Nrr Unspecified

Specimen Details

Sample ID:	krcc2	Voucher Status:	
Process ID:	TDB020-20	Tissue Descriptor:	
Project:	TDB	Sex:	
Institution Storing:	Regional Agricultural Research Station, Pattambi	Reproduction:	
		Life Stage:	Adult
		Extra Info:	Sex: u
		Associated Taxa:	
Field ID:	Koranchira	Associated Specimens:	
Museum ID:			
Collection Code:			
Reference Link:			
Note:			

Taxonomy

Phylum:	Arthropoda	Identification:	Thrips hawaiiensis (Morgan, 1913)
Class:	Insecta		
Order:	Thysanoptera	Rank:	Species
Family:	Thripidae	Identifier:	
Subfamily:	Thripinae	Identification Method:	Morphological
Genus:	Thrips	Identifier Institution:	
Species:	Thrips hawaiiensis	Identifier Email:	
		Taxonomy Note:	

Process ID:	TDB020-20
Identification:	Thrips hawaiiensis (Morgan, 1913)
Identified by	
Collected in	India, Kerala
by	Namitha T.H.
Institution Storing:	Regional Agricultural Research Station, Pattambi
Field ID:	Koranchira
Museum ID:	

Collection Data

Country:	India	Collector:	Namitha T.H.
Province/State:	Kerala	Date Collected:	02-Feb-2020
Region/County:	Koranchira	Date Accuracy:	
Sector:		Time Collected:	
Exact Site:		Site Code:	
Lat/Lon:		Habitat:	
Elevation:		Sampling Protocol:	
Elevation Accuracy:		Coord. Source:	
Depth:		Coord. Accuracy	
Depth Accuracy:			
Collection Event ID:			
Collection Notes:			

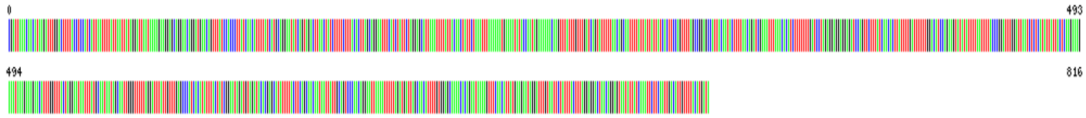
Specimen Details Current

Sample ID:	krcc2
Process ID:	TDB020-20
Project:	TDB
Tax Names:	Arthropoda, Insecta, Thysanoptera, Thripidae, Thripinae, Thrips, Thrips hawaiiensis
Taxon:	Thrips hawaiiensis
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Animalia

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
COI-5P	817	28%	0%	1

Illustrative Barcode



Nucleotide Sequence

CGATTAAACAACATAAGATTTGGACTTTTACCTCCATCATTAATTTTATTAATTATAGGATTAATAAAAGAAGGAGCAGGAACAGGATGAACAGTTTATCCACCCTTATCAACATTTTA
TCATGCAGGAATATCAGTAGACTTAACTATCTTTCTTTACACTTAGCAGGAATTTCATCTATTCTAGGAGCATTAAATTTTATTACAACTATTCTAAATTTAAAAAATGAAAATATAC
CAATAGAAAAAACAAGTTTATTTGTTTGATCAGTATTTTTAACAGCAATTTTATTATTATTATCACTTCCAGTTTTAGCCGGAGCCATTACAATACTTTTAACAGATCGAAATTTAAAC
ACATCTTTTTTTGATCCTAGAGGAGGAGGAGATCCAGTTCTTTATCAACACTTATTTTGATTTTTTGGACATCCAGAAGTATATATTTTAATTTTACCCGGATTTGGATTAATCTCTCA
TATTATTACTCAAGAAAGAAATAAAGAAAGAACATTTGGTTTACTAGGAATAATTTATGCAATAATAGCAATTGGGTTTTTAGGATTTATTGTTTGAGCCCATCACATATTTACTATCG
GAATAGATGTAGATACACGAGCATATTTTACTTCAGCAACAATAATTATTGCAGTCCCAACAGGAATTAAAATTTTTAGATGATTAGCAACATTTTGTGGAGCAAAACACAGAATAAAA
GTTTCAACATTATGAAGAATAGGATTTGTAATTCTATTTACTTTAGGAGGACTAACAGGAGTAATATTATCAAATTCTTCAATTGACATTGTTTACATGATA

Amino Acid Sequence

RLNNMSFGLLPPSLILLIMGLMKEGAGTGWTVYPPLSTFYHAGMSVDLTIFSLHLAGISSILGALNFITTILNLKNENMPMEKTSLFVWSVFLTAILLLLSLPVLAGAITMLLTDRNLN
TSFFDPSGGGDPVLYQHLFWFFGHPEVYILILPGFGLISHIITQESNKESTFGLLGMIYAMMAIGFLGFIVWAHHMFTIGMDVDTRAYFTSATMIIAVPTGIKIFSWLATFCGAKHSMK
VSTLWSMGFVILFTLGGLTGVMLSNSSIDIVLHDX

Sequence Metadata

Genbank Accession:	MT793721
Translation Matrix:	Invertebrate Mitochondrial
Last Updated:	2020-07-17 11:20:34.937614
Sequence Runsite:	Kerala Agricultural University

Modify Sequence:

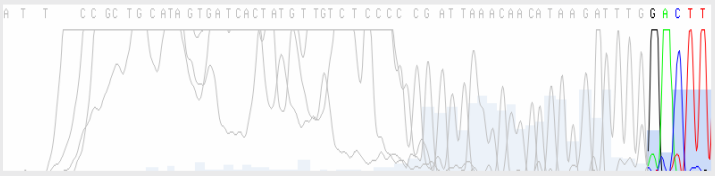
Clear Sequence | Edit Sequence

Identify Sequence:

Full DB | Species DB | Published DB | Full Length DB

Download Trace

Sequencing Date	2020-07-06 21:37:45
Trace Direction	F
Forward Primer	UBC6 F
Reverse Primer	R COI
Sequence Primer	UBC6 F
Status	high qual
Trace Runsite	Kerala Agricultural University



Trace Tags

Trace Comments

New Comment

kkcc1 - DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala [TDB]

Specimen Details

Sample ID:	kkcc1	**Voucher Status:**	
Process ID:	TDB009-20	**Tissue Descriptor:**	
Project:	TDB	**Sex:**	
Institution Storing:	Regional Agricultural Research Station, Pattambi	**Reproduction:**	
		Life Stage:	Adult
		Extra Info:	Sex: u
Field ID:	Kattakampal	**Associated Taxa:**	
Museum ID:		**Associated Specimens:**	
Collection Code:			
Reference Link:			
Note:			

Taxonomy

Phylum:	Arthropoda	**Identification:**	Ayyaria chaetophora
Class:	Insecta		
Order:	Thysanoptera	**Rank:**	Species
Family:	Thripidae	**Identifier:**	
Subfamily:	Thripinae	**Identification Method:**	Morphological
Genus:	Ayyaria	**Identifier Institution:**	
Species:	Ayyaria chaetophora	**Identifier Email:**	
		Taxonomy Note:	

Process ID:	TDB009-20
Identification:	Ayyaria chaetophora
Identified by	
Collected in	India, Kerala
by	Namitha T.H
Institution Storing:	Regional Agricultural Research Station, Pattambi
Field ID:	Kattakampal
Museum ID:	

Collection Data

Country:	India	**Collector:**	Namitha T.H
Province/State:	Kerala	**Date Collected:**	25-Dec-2019
Region/County:	Kattakampal	**Date Accuracy:**	
Sector:		**Time Collected:**	
Exact Site:		**Site Code:**	
Lat/Lon:		**Habitat:**	
Elevation:		**Sampling Protocol:**	
Elevation Accuracy:		**Coord. Source:**	
Depth:		**Coord. Accuracy**	
Depth Accuracy:			
Collection Event ID:			
Collection Notes:			

Specimen Details Current

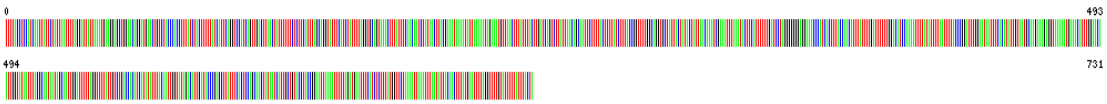
Sample ID:	kkcc1
Process ID:	TDB009-20
Project:	TDB
Tax Names:	Arthropoda, Insecta, Thysanoptera, Thripidae, Thripinae, Ayyaria, Ayyaria chaetophora
Taxon:	Ayyaria chaetophora
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Animalia

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
COI-5P	732	27.6%	0%	1

COI-5P

Illustrative Barcode



Nucleotide Sequence

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AACTATTTTTTCTCTTCATTTAGCAGGTATTTCATCTATTTTAGGTGCAGTAAACTTTATTTCAACAATCTATAACTTACGAAATAAAATATTAAAAATAGAAATGTTAACTTTATTT
ACTTGATCAGTCTTATTAACAGCAATTCTTTTACTTTTATCTTTACCGGTTTTGGCAGGTGCTATTACTATACTATTAACAAATCGAAATTTAAATACTTCATTTTTTGATCCTAGGG
GGGGAGGAAACCCATCACTTTACCAACATTTATTTTGATTTTTTGGACATCCAAAATTTTATATTTTAATTTTACCCGGATTTGGAATAATTTCACATATTGTAATGCAAGAAAGAGG
TAAAAATGAAACATTTGGAACAATTGGTATAATTTACGCAATAATAGCAATTGGATTTTTAGGTTTTATTGTCTGACCACATCATATATTTACAATTGGTATAGACATTGATACCCGA
GCTTATTTCACTCCAGCAACTATAATCATTGCTGTACCTACCGGAATTAAAATTTTTAGATGAATTTCATCTCTTTATGGTTCTAAAATTAAATTTAATATTACAACTTTATGAATTT
TAGGTTTTGTATTTTTATTTACTA

Amino Acid Sequence

LPPSIILLILGLLNEGAGTGWTVYPPLSTFVHSGPSVDLTIFSLHLAGISSILGAVNFISTIYNLRNKMLKMEMLTLFTWSVLLTAILLLLSLPVLAGAITMLLTNRNLNTSFFDPSG
GGNPSLYQHLFWFFGHPKFYILILPGFGMISHIVMQESGKNETFGTIGMIYAMMAIGFLGFIVWPHHMFTIGMDIDTRAYFTPATMIIAVPTGIKIFSWISSLYGSKIKFNITTLWIL
GFVFLFTX

Sequence Metadata

Genbank Accession:	MT821343
Translation Matrix:	Invertebrate Mitochondrial
Last Updated:	2020-07-17 10:47:46.04632
Sequence Runsite:	Kerala Agricultural University

Modify Sequence:

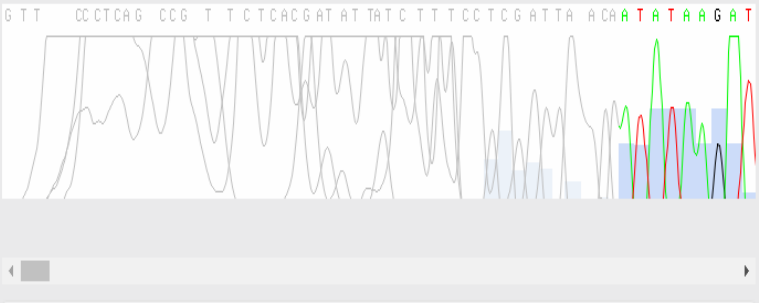
Clear Sequence | Edit Sequence

Identify Sequence:

Full DB | Species DB | Published DB | Full Length DB

Download Trace

Sequencing Date	2020-06-23 09:57:57
Trace Direction	F
Forward Primer	UBC6 F
Reverse Primer	R COI
Sequence Primer	UBC6 F
Status	med qual
Trace Runsite	Kerala Agricultural University



vkcu1 - DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala [TDB]

Specimen Details

Sample ID:	vkcu1	**Voucher Status:**	
Process ID:	TDB022-20	**Tissue Descriptor:**	
Project:	TDB	**Sex:**	
Institution Storing:	Regional Agricultural Research Station, Pattambi	**Reproduction:**	
		Life Stage:	Adult
		Extra Info:	Sex: u
Field ID:	Vellanikkara	**Associated Taxa:**	
Museum ID:		**Associated Specimens:**	
Collection Code:			
Reference Link:			
Note:			

Collection Data

Country:	India	**Collector:**	Namitha T.H.
Province/State:	Kerala	**Date Collected:**	02-Feb-2020
Region/County:	Vellanikkara	**Date Accuracy:**	
Sector:		**Time Collected:**	
Exact Site:		**Site Code:**	
Lat/Lon:		**Habitat:**	
Elevation:		**Sampling Protocol:**	
Elevation Accuracy:		**Coord. Source:**	
Depth:		**Coord. Accuracy**	
Depth Accuracy:			
Collection Event ID:			
Collection Notes:			

Process ID: TDB022-20
Identification: Ayyaria chaetophora
Identified by
Collected in India, Kerala
by Namitha T.H.
Institution Storing: Regional Agricultural Research Station, Pattambi
Field ID: Vellanikkara
Museum ID:

Taxonomy

Phylum:	Arthropoda	**Identification:**	Ayyaria chaetophora
Class:	Insecta	**Rank:**	Species
Order:	Thysanoptera	**Identifier:**	
Family:	Thripidae	**Identification Method:**	Morphological
Subfamily:	Thripinae	**Identifier Institution:**	
Genus:	Ayyaria	**Identifier Email:**	
Species:	Ayyaria chaetophora	**Taxonomy Note:**	

Specimen Details Current

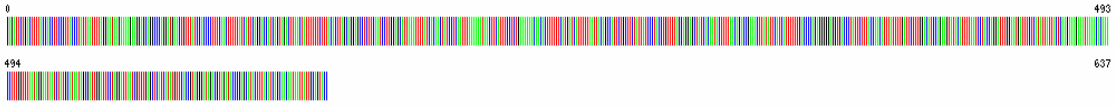
Sample ID:	vkcu1
Process ID:	TDB022-20
Project:	TDB
Tax Names:	Arthropoda, Insecta, Thysanoptera, Thripidae, Thripinae, Ayyaria, Ayyaria chaetophora
Taxon:	Ayyaria chaetophora
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Animalia

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
COI-5P	638	34.6%	0%	1

COI-5P *

Illustrative Barcode



Nucleotide Sequence

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CAACACTTTTTTCATGATCATTTTTTAATTACAGCCATTCTGCTTCTATTATCCCTACCTGTTTTAGCAGGAGCTATCACAATACTTTTAACAGACCGAAACTTATATTCATCATTTTT
TGACCCCAGAGGGGGAGGTGACCCTATTCTTTACCAACATTTATTTTGATTCTTTGGACATCCAGAAGTATACATTTTAATCCTACCAGGATTTGGAATGATTTCTCATATTATTACT
CAAGAAAGAAATAAAACACAAACCTTTGGTTTAATAGGAATAATCTATGCATAATAGCGATTGGATTCCTAGGATTAATCGTTTGAGCTCATCATATGTTCACCGTAGGGATAGACGT
AGATACCCGAGCATACTTTACATCAGCAACAATAATTATTGCAGTACC

Amino Acid Sequence

EILTFTTFPYPINFSNKKSSSSPSMNCLSTPFNFLSFSTISSLNYFFFTLSSSLFNPWSIKFYHNYPQFKK*LHFLWKNNTFFMIIFNYSHSASIIPTCFSSSYHNTFNSPKLMFIIF
WPQSGSWPYSLPTFILILWTSSSMHFNPTSIWNDFSYYYSSKK*NTNLWFNSNNLCMMAIGFLGLIVWAHHMFTVGMDVDTRAYFTSATMIIAVX

Sequence Metadata

Genbank Accession:	MT826856
Translation Matrix:	Invertebrate Mitochondrial
Last Updated:	2020-07-17 11:20:34.965556
Sequence Runsite:	Kerala Agricultural University

Modify Sequence:

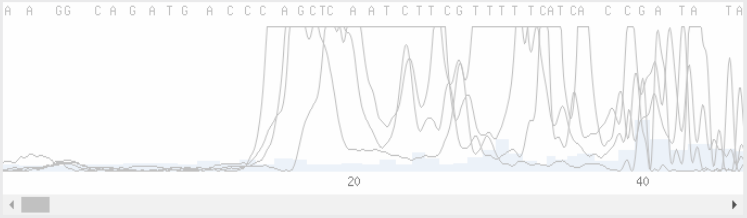
Clear Sequence | Edit Sequence

Identify Sequence:

Full DB | Species DB | Published DB | Full Length DB

Download Trace

Sequencing Date	2020-07-06 21:37:45
Trace Direction	F
Forward Primer	UBC6 F
Reverse Primer	R COI
Sequence Primer	UBC6 F
Status	med qual
Trace Runsite	Kerala Agricultural University



Trace Tags

Trace Comments

New Comment +

hhto1 - DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala [TDB]

Sample ID:	hhto1	**Voucher Status:**	
Process ID:	TDB025-20	**Tissue Descriptor:**	
Project:	TDB	**Sex:**	
Institution Storing:	Regional Agricultural Research Station, Pattambi	**Reproduction:**	
		Life Stage:	Adult
		Extra Info:	Sex: u
Field ID:	Hightech horticulture	**Associated Taxa:**	
		Associated Specimens:	
Museum ID:			
Collection Code:			
Reference Link:			
Note:			

Taxonomy

Phylum:	Arthropoda	**Identification:**	Thrips palmi (Karny, 1925)
Class:	Insecta	**Rank:**	Species
Order:	Thysanoptera	**Identifier:**	
Family:	Thripidae	**Identification Method:**	Morphological
Subfamily:	Thripinae	**Identifier Institution:**	
Genus:	Thrips	**Identifier Email:**	
Species:	Thrips palmi	**Taxonomy Note:**	

Process ID:	TDB025-20
Identification:	Thrips palmi (Karny, 1925)
Identified by	
Collected in	India, Kerala
by	Namitha T.H.
Institution Storing:	Regional Agricultural Research Station, Pattambi
Field ID:	Hightech horticulture
Museum ID:	

Collection Data

Country:	India	**Collector:**	Namitha T.H.
Province/State:	Kerala	**Date Collected:**	02-Apr-2020
Region/County:	Hightech horticulture	**Date Accuracy:**	
		Time Collected:	
Sector:		**Site Code:**	
Exact Site:		**Habitat:**	
Lat/Lon:		**Sampling Protocol:**	
Elevation:		**Coord. Source:**	
Elevation Accuracy:		**Coord. Accuracy**	
Depth:			
Depth Accuracy:			
Collection Event ID:			
Collection Notes:			

Specimen Details Current

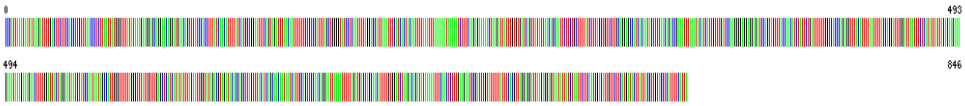
Sample ID:	hhto1
Process ID:	TDB025-20
Project:	TDB
Tax Names:	Arthropoda, Insecta, Thysanoptera, Thripidae, Thripinae, Thrips, Thrips palmi
Taxon:	Thrips palmi
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Animalia

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
COI-5P	847	30.3%	0%	1

COI-5P *

Illustrative Barcode



Nucleotide Sequence

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ATATTAACAGCAATTCTTCTGCTTTTATCTTTACCTGTTCTTGCAGGAGCCATCACAATACTTTTAACAGACCGAAATTTAAATACATCATTCTTTGATCCAAGGGGAGGAGGAGATCCAGTACTTTACCAACATCT
ATTCTGATTTTTTGGTCACCCAGAGGTTTACATTTTAATTTTACCAGGATTTGGATTAATTTCTCATATTATTACACAAGAAAGAAATAAAGAAAGAACTTTTGGATTACTAGGAATAATCTATGCAATAATAGCCA
TTGGGTTTTTAGGCTTTATTGTTTGAGCTCATCATATATTTACAATTGGTATAGACGTAGATACACGAGCTTACTTTACATCAGCAACAATAATCATTGCAGTTCCAACAGGAATTAAAATTTTAGATGACTAGCA
ACATTTTGTGGATCTAAGACAGATATAAAAATTTCAACTCTTTGAAGAATCGGATTTGTATTTTATTTCACGTTAGGAGGATTAACAGGAGTAATATTATCTAATTCTTCAATCGACATTGTTCTTCATGATACTT
ATTACGTTGTAGCTCATTTTCATTA
```

Amino Acid Sequence

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RLNNMSFWLLPPSLTLLIMGLYKEGAGTGWTVYPPLSTFYHAGISVDLTIFSLHLAGVSSILGALNFITTILNLKNKNLSSEKLSLFVWSVMLTAILLLLSLPVLAGAITMLLTDRNLNTSFFDPSGGGDPVLYQHL
FWFFGHPEVYILILPGFGLISHIITQESNKESTFGLLGMIYAMMAIGFLGFIVWAHHMFTIGMDVDTRAYFTSATMIIAVPTGIKIFSWLATFCGSKTDMKISTLWSIGFVFLFHVSSINSSNII*FFNRHCSSWYL
LRCSSFSL
```

Sequence Metadata

Genbank Accession:	MT792799
Translation Matrix:	Invertebrate Mitochondrial
Last Updated:	2020-07-17 11:20:35.0062
Sequence Runsite:	Kerala Agricultural University

Modify Sequence:

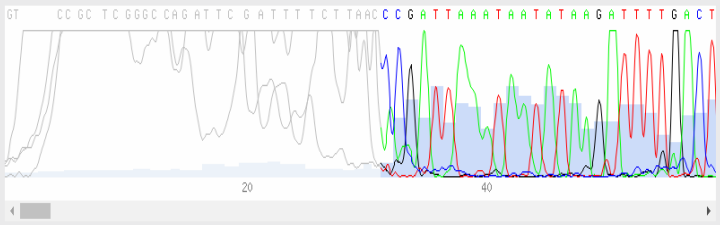
Clear Sequence | Edit Sequence

Identify Sequence:

Full DB | Species DB | Published DB | Full Length DB

Download Trace

Sequencing Date	2020-07-06 21:37:45
Trace Direction	F
Forward Primer	UBC6 F
Reverse Primer	R COI
Sequence Primer	UBC6 F
Status	high qual
Trace Runsite	Kerala Agricultural University



Trace Tags

Trace Comments

New Comment +

cyci1 - DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala [TDB]

Specimen Details

Sample ID:	cyci1	**Voucher Status:**	
Process ID:	TDB013-20	**Tissue Descriptor:**	
Project:	TDB	**Sex:**	
Institution Storing:	Regional Agricultural Research Station, Pattambi	**Reproduction:**	
		Life Stage:	Adult
		Extra Info:	Sex: u
Field ID:	Chalissery	**Associated Taxa:**	
Museum ID:		**Associated Specimens:**	
Collection Code:			
Reference Link:			
Note:			

Taxonomy

Phylum:	Arthropoda	**Identification:**	Thrips florum (Schmutz, 1913)
Class:	Insecta	**Rank:**	Species
Order:	Thysanoptera	**Identifier:**	
Family:	Thripidae	**Identification Method:**	Morphological
Subfamily:	Thripinae	**Identifier Institution:**	
Genus:	Thrips	**Identifier Email:**	
Species:	Thrips florum	**Taxonomy Note:**	

Process ID:	TDB013-20
Identification:	Thrips florum (Schmutz, 1913)
Identified by	
Collected in	India, Kerala
by	Namitha T.H.
Institution Storing:	Regional Agricultural Research Station, Pattambi
Field ID:	Chalissery
Museum ID:	

Collection Data

Country:	India	**Collector:**	Namitha T.H.
Province/State:	Kerala	**Date Collected:**	02-Feb-2020
Region/County:	Chalissery	**Date Accuracy:**	
Sector:		**Time Collected:**	
Exact Site:		**Site Code:**	
Lat/Lon:		**Habitat:**	
Elevation:		**Sampling Protocol:**	
Elevation Accuracy:		**Coord. Source:**	
Depth:		**Coord. Accuracy**	
Depth Accuracy:			
Collection Event ID:			
Collection Notes:			

Specimen Details Current

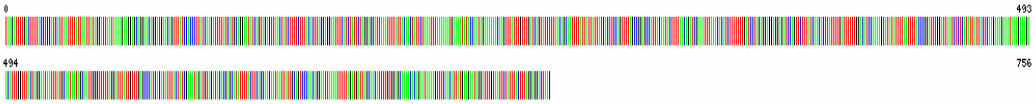
Sample ID:	cyci1
Process ID:	TDB013-20
Project:	TDB
Tax Names:	Arthropoda, Insecta, Thysanoptera, Thripidae, Thripinae, Thrips, Thrips florum
Taxon:	Thrips florum
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Animalia

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
COI-5P	757	27.9%	0%	1

COI-5P

Illustrative Barcode



Nucleotide Sequence

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TTTATTGTTTGAGCCCATCACATATTTACTATCGAAATAGATGTAGATACACGAGCATATTTTACTTCAGCAACAATAATTATTGCAGTCCCAACAGGAATTAAAATTTTTAGATGATTAGCAACATTTTGTGGAGC
AAAACACAGAATAAAAGTTTCAACATTATGAAGAATAGGATTTGTAATTCTATTTACTTTAGGAGGACTAAC
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Amino Acid Sequence

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HPKVYILILPGFGLISHIITQESNKESTFGLLGMIYAMMAIGFLGFIVWAHHMFTIEMDVDTRAYFTSATMIIAVPTGIKIFSWLATFCGAKHSMKVSTLWSMGFVILFTLGGLX
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Sequence Metadata

Genbank Accession:	MT765068
Translation Matrix:	Invertebrate Mitochondrial
Last Updated:	2020-07-17 10:47:46.108964
Sequence Runsite:	Kerala Agricultural University

Modify Sequence:

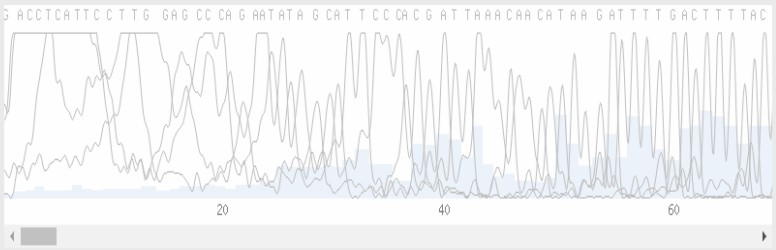
Clear Sequence | Edit Sequence

Identify Sequence:

Full DB | Species DB | Published DB | Full Length DB

Download Trace

Sequencing Date	2020-03-11 20:41:41
Trace Direction	F
Forward Primer	UBC6 F
Reverse Primer	R COI
Sequence Primer	UBC6 F
Status	low qual
Trace Runsite	Kerala Agricultural University



Trace Tags

Trace Comments

New Comment

hhbr1 - DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala [TDB]

Specimen Details

Sample ID:	hhbr1	**Voucher Status:**	
Process ID:	TDB015-20	**Tissue Descriptor:**	
Project:	TDB	**Sex:**	
Institution Storing:	Regional Agricultural Research Station, Pattambi	**Reproduction:**	
		Life Stage:	Adult
		Extra Info:	Sex: u
		Associated Taxa:	
Field ID:	Hightech horticulture	**Associated Specimens:**	
Museum ID:			
Collection Code:			
Reference Link:			

Collection Data

Country:	India	**Collector:**	Namitha T.H.
Province/State:	Kerala	**Date Collected:**	02-Feb-2020
Region/County:	Hightech horticulture	**Date Accuracy:**	
		Time Collected:	
Sector:		**Site Code:**	
Exact Site:		**Habitat:**	
Lat/Lon:		**Sampling Protocol:**	
Elevation:		**Coord. Source:**	
Elevation Accuracy:		**Coord. Accuracy**	
Depth:			
Depth Accuracy:			
Collection Event ID:			
Collection Notes:			

Taxonomy

Process ID:	TDB015-20
Identification:	Thrips palmi (Karny, 1925)
Identified by	
Collected in	India, Kerala
by	Namitha T.H.
Institution Storing:	Regional Agricultural Research Station, Pattambi
Field ID:	Hightech horticulture
Museum ID:	

Phylum:	Arthropoda	**Identification:**	Thrips palmi (Karny, 1925)
Class:	Insecta	**Rank:**	Species
Order:	Thysanoptera	**Identifier:**	
Family:	Thripidae	**Identification Method:**	Morphological
Subfamily:	Thripinae	**Identifier Institution:**	
Genus:	Thrips	**Identifier Email:**	
Species:	Thrips palmi	**Taxonomy Note:**	

Specimen Details Current

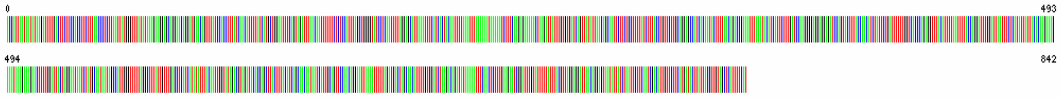
Sample ID:	hhbr1
Process ID:	TDB015-20
Project:	TDB
Tax Names:	Arthropoda, Insecta, Thysanoptera, Thripidae, Thripinae, Thrips, Thrips palmi
Taxon:	Thrips palmi
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Animalia

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
COI-5P	843	30.2%	0%	1

COI-5P

Illustrative Barcode



Nucleotide Sequence

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AATACATCATTCTTTGATCCAAGGGGAGGAGGAGATCCAGTACTTTACCAACATCTATTCTGATTTTTTGGTCACCCAGAGGTTTACATTTTAATTTTACCAGGATTTGGATTAATTT
CTCATATTATTACACAAGAAAGAAATAAAGAAAGAACTTTTGGATTACTAGGAATAATCTATGCAATAATAGCCATTGGGTTTTTAGGCTTTATTGTTTGAGCTCATCATATATTTAC
AATTGGTATAGACGTAGATACACGAGCTTACTTTACATCAGCAACAATAATCATTGCAGTTCCAACAGGAATTAAAATTTTAGATGACTAGCAACATTTTGTGGATCTAAGACAGAT
ATAAAAATTTCAACTCTTTGAAGAATCGGATTTGTATTTTATTTACGTTAGGAGGATTAACAGGAGTAATATTATCTAATTCTTCAATCGACATTGTTCTTCATGATACTTATTACG
TTGTAGCTCATTTTCAT
```

Amino Acid Sequence

```
TINNMSFWLLPPSLTLLIMGLYKEGAGTGWTVYPPLSTFYHAGISVDLTIFSLHLAGVSSILGALNFITTILNLKNKNLSSEKLSLFVWSVMLTAILLLLSLPVLAGAITMLLTDRNL
NTSFFDPSGGGDPVLYQHLFWFFGHPEVYILILPGFGLISHIITQESNKESTFGLLGMIYAMMAIGFLGFIVWAHHMFTIGMDVDTRAYFTSATMIIAVPTGIKIFSWLATFCGSKTD
MKISTLWSIGFVFLFTLGGLTGVMLSNSSIDIVLHDTYYVVAHFH
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Sequence Metadata

Genbank Accession:	MT780221
Translation Matrix:	Invertebrate Mitochondrial
Last Updated:	2020-07-17 11:20:34.861233
Sequence Runsite:	Kerala Agricultural University

Modify Sequence:

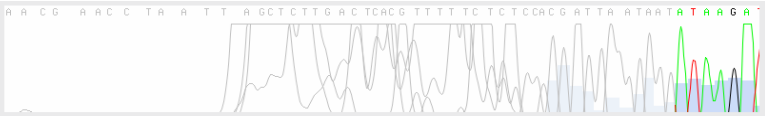
Clear Sequence | Edit Sequence

Identify Sequence:

Full DB | Species DB | Published DB | Full Length DB

Download Trace

Sequencing Date	2020-06-23 09:57:57
Trace Direction	F
Forward Primer	UBC6 F
Reverse Primer	R COI
Sequence Primer	UBC6 F
Status	high qual
Trace Runsite	Kerala Agricultural University



Trace Tags

Trace Comments

New Comment

cycp4 - DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala [TDB]

Specimen Details

Sample ID:	cycp4	**Voucher Status:**	
Process ID:	TDB019-20	**Tissue Descriptor:**	
Project:	TDB	**Sex:**	
Institution Storing:	Regional Agricultural Research Station, Pattambi	**Reproduction:**	
		Life Stage:	Adult
		Extra Info:	Sex: u
		Associated Taxa:	
Field ID:	Chalissery	**Associated Specimens:**	
Museum ID:			
Collection Code:			
Reference Link:			
Note:			

Taxonomy

Phylum:	Arthropoda	**Identification:**	Ayyaria chaetophora
Class:	Insecta	**Rank:**	Species
Order:	Thysanoptera	**Identifier:**	
Family:	Thripidae	**Identification Method:**	Morphological
Subfamily:	Thripinae	**Identifier Institution:**	
Genus:	Ayyaria	**Identifier Email:**	
Species:	Ayyaria chaetophora	**Taxonomy Note:**	

Process ID:	TDB019-20
Identification:	Ayyaria chaetophora
Identified by	
Collected in	India, Kerala
by	Namitha T.H.
Institution Storing:	Regional Agricultural Research Station, Pattambi
Field ID:	Chalissery
Museum ID:	

Collection Data

Country:	India	**Collector:**	Namitha T.H.
Province/State:	Kerala	**Date Collected:**	02-Feb-2020
Region/County:	Challisery	**Date Accuracy:**	
Sector:		**Time Collected:**	
Exact Site:		**Site Code:**	
Lat/Lon:		**Habitat:**	
Elevation:		**Sampling Protocol:**	
Elevation Accuracy:		**Coord. Source:**	
Depth:		**Coord. Accuracy**	
Depth Accuracy:			
Collection Event ID:			
Collection Notes:			

Specimen Details Current

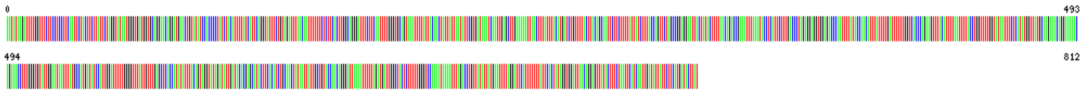
Sample ID:	cycp4
Process ID:	TDB019-20
Project:	TDB
Tax Names:	Arthropoda, Insecta, Thysanoptera, Thripidae, Thripinae, Ayyaria, Ayyaria chaetophora
Taxon:	Ayyaria chaetophora
Rank Name:	species
Sampling Protocol:	N/A
BIN URI:	N/A
Kingdom:	Animalia

Marker Summary

Marker Code	Sequence Length	GC	Ambiguous	Trace Count
COI-5P	813	30.5%	0%	1

COI-5P

Illustrative Barcode



Nucleotide Sequence

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GCAAGAAACAGGAAAAACAGAAACCTTTGGGTGTATTGGAATAATTTATGCCATGATATCCATTGGATTTTTGGGGTTTATTGTTTGAGCTCACCATATATTTACAGTAGGTATAGATA
TTGATACACGAGCATACTTCACATCTGCAACTATAATTATTGCTGTACCAACAGGGATTAAAATTTTTAGATGATTGGCTACTCTTTTTGGGTCCAAAATAAAATTTAGACCTGTTATA
TTATGAATTTTAGGATTCATTTTTTTATTCACTATAGGTGGATTAACAGGAGTTATACTTTCTAATTCATCAATTGATATTATTCTTCATGATACTTAT

Amino Acid Sequence

NMSFWFLPPSILLLILGMMGDGAGTGWTVYPPLSTFYHSGISVDLTIFSLHLAGISSILGAVNFITTIINIRDKSIELEKLTLFTWSVLLTAILLLLSLPVLAGGITMLLTDRNLNTSF
FDPSGGGDPILYQHLFWFFGHPEVYILILPGFGMISHMVMQETGKTETFGCIGMIYAMMSIGFLGFIVWAHHMFTVGMDIDTRAYFTSATMIIAVPTGIKIFSWLATLFGSKMKFSPVM
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Sequence Metadata

Genbank Accession:	MT821303
Translation Matrix:	Invertebrate Mitochondrial
Last Updated:	2020-07-17 11:20:34.92281
Sequence Runsite:	Kerala Agricultural University

Modify Sequence:

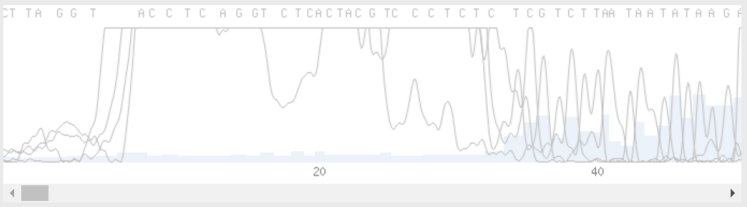
Clear Sequence | Edit Sequence

Identify Sequence:

Full DB | Species DB | Published DB | Full Length DB

Download Trace

Sequencing Date	2020-07-06 21:37:45
Trace Direction	F
Forward Primer	UBC6 F
Reverse Primer	R COI
Sequence Primer	UBC6 F
Status	failed
Trace Runsite	Kerala Agricultural University



Trace Tags

Trace Comments

New Comment

Table . List of accession numbers and process ID generated for the sequences deposited in NCBI and BOLD respectively.

Sl. No	Sample ID	Locus	Accession Number	Process ID
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2.	Kkbr3	COI	MT775824	TDB008-20
3.	Kkcc1	COI	MT821343	TDB009-20
4.	Pnci2	COI	MT765069	TDB010-20
5.	Pmcp2	COI	MT826968	TDB011-20
6.	Vkci3	COI	MT510646	TDB012-20
7.	Cyci1	COI	MT765068	TDB013-20
8.	Kubr2	COI	MT775825	TDB014-20
9.	Hhbr1	COI	MT780221	TDB015-20
10.	Vvbr3	COI	MT780222	TDB016-20
11.	Kkcp1	COI	MT826967	TDB017-20
12.	Vvcp3	COI	MT821304	TDB018-20
13.	Cycp4	COI	MT821303	TDB019-20
14.	Krcc2	COI	MT793721	TDB020-20
15.	Pncc3	COI	MT792798	TDB021-20
16.	Vkcu1	COI	MT826856	TDB022-20
17.	Vvcu2	COI	MT826857	TDB023-20
18.	Cycu3	COI	MT826858	TDB024-20
19.	Hhto1	COI	MT792799	TDB025-20
20.	Cyto2	COI	MT792800	TDB026-20
21.	Vvto3	COI	MT792801	TDB027-20

5. DISCUSSION

Thrips is regarded as a notorious plant pest under the family Thripidae, generally considered under the order Thysanoptera. The two suborders of Thysanoptera are Terebrantia and Tubulifera which could be discriminated by morphological, behavioural, and developmental characteristics (Mound et al., 2005)

Among 6,000 known thrips species, only a few have been catalogued as pollinator but most of them functioning as predator, pest, and vector for plant viruses (Lewis 1997, Pappu *et al*., 2009). Majority bring about direct damage to crops and a few act as Tospovirus vector (genus Tospovirus, family Bunyaviridae) which could make huge impact on vegetables, fiber and ornamental crops by transmission of viral diseases (Whitfield *et al*., 2005).

Larger number of species shown cryptic characteristics which turns into taxonomic identification difficult. Molecular level identification could be the most acceptable method to get rid of this crisis (Rubinoff *et al*., 2006).

Molecular taxonomy or DNA barcoding is the most innovative molecular method to differentiate known and unknown species on the basis of nucleotide sequence in a DNA fragment (Hebert *et al*., 2003). DNA barcoding is the utilization of a short standardized DNA segment of 658 bp from the mitochondrial cytochrome c oxidase COX I) gene to ascertain unknown species. This study was conducted to generate DNA barcodes for different species belonging to thripidae in selected vegetable crops like chilli, cucumber, cowpea, coccinia, brinjal, tomato and to study the variability among them.

5.1. Purposive survey and identification

Thrips infested leaf samples were collected from different locations of Thrissur and Palakkad districts of Kerala from the six selected vegetable crops like cucumber, coccinia, chilli, cowpea, brinjal and tomato individually in polythene bags. Abundant sample collection made possible during afternoon because thrips

mostly preferred to live in warmer climatic conditions. Thrips specimens from each crop were kept separately in 70% and 100% ethanol for taxonomic and molecular purposes. The taxonomic identification was done at Zoological Survey of India, Kolkata. The four thrips species, *T.palmi*, *T.florum*, *T. hawaiiensis* and*Ayyaria chaetophora* were obtained from 21 samples of thrips from vegetable crops. Thrips stored in 100% ethanol at -20 ^{0}C were used for DNA extraction. The species *T. hawaiiensis* and *A. chaetophora* were found infesting the vegetable crop coccinia. Likewise, *A. chaetophora* and *T. palmi* were found infesting the vegetable crops brinjal and tomato. *T. palmi* was the dominant thrips species infesting in greenhouse vegetable crops. Sakimura *et al*. (1986) reported *T. palmi* as a serious pest causing acute damages to the economical vegetable crops like brinjal, pepper, potato, tobacco, cucumber, melons, squash and cowpea which have been reported from Asia.

5.2. Molecular study

The candidate loci used in the study was mitochondrial cytochrome oxidase I (mtCOI) which is the predominant marker for species identification in the animal kingdom (Hebert *et al*., 2003). COI primer is the most suitable molecular marker for species level identification within the genus thrips, because it exhibits more consistent interspecific variation than other markers (Glover *et al*., 2010).

Tyagi *et al*. (2008) used individual specimen of each species of thrips by employing a non-destructive method of DNA extraction using QIAamp DNA Mini Kit (Qiagen, Valencia, CA) by making an intersegmental abdominal cut to each specimen, and these were lysed overnight at 56 °C in buffer ATL with proteinase K. Non-destructive method of DNA extraction is regarded as the most easy, convenient and accurate method of insect DNA isolation.

In the present study mitochondrial cytochrome oxidase I (COI) primer was used for identification of thrips specimens collected from different vegetable crops. DNA isolation of thrips specimens were conducted by using QIAGEN DNeasy blood and tissue kit by following the kit protocol. A small cut was made to each specimen before overnight incubation at 60 °C in buffer ATL with proteinase K for tissue lysis.

The kit protocol preferred the tissue lysis temperature as 56 °C but after increasing the temperature to 60 °C resulted in good quality DNA. Individual thrips were taken to avoid mixing of distinct genotypes.

5.2.1. Quality assessment of isolated DNA

Quality assessment of isolated DNA was carried out by using UV–Visible spectrophotometer. DNA absorbs light actively at 260 nm whereas proteins shows absorbance maxima at 280 nm. The purity of DNA was computed as the ratio of absorbance at 260 nm divided to the reading at 280 nm. The samples with the ratio between 1.8 and 2.0 indicated as good quality samples and majority of samples obtained in this range.

5.2.2. PCR amplification and sequencing

Good quality total genomic DNA was used for the PCR amplification of CO1 loci by using the previously reported primers UBC6 F (forward) and R COI (reverse). The CO1 marker from mitochondrial cytochrome C Oxidase subunit I gene, were used for generating species specific signature sequences from mitochondrial genome. The PCR assay was standardized by changing the annealing temperature and obtained adequate results. Amplified products were visualized by 2 per cent agarose gel stained with ethidium bromide and obtained clear bands at a size range of 890 bp. A total of 21 sequences of thrips specimens were amplified with COI primers and sequencing was carried out at AgriGenome Lab. Pvt. Ltd., Cochin. Column purification was done before sequencing to prevent further contamination.

5.2.3. Analysis of sequence homology using BLASTn

BLAST is a sequence homology analysis tool for comparing primary biological sequence information, such as the amino-acid sequences of protein or the nucleic acid base pairs to align these databases in terms of identity level that range from perfect matches to very low similarity. It displays the query results in order of decreasing significance, and in the form of graphics, tables, and alignments (Altschul *et al.*,1990). BLAST compute a measure based on precise mutation scores. Checking

the alignment of sequences using BLASTn made the structure appropriate for further evaluation. BLASTn sequence analysis of thrips species, *T. florum*, *T. palmi*, *T. hawaiiensis* and *A. chaetophora* having range of similarity from very high similarity to low level similarity with respect to our sequences were observed.

5.2.4. Barcode gap analysis

Sequences of 21 thrips specimens were readily aligned using clustalW tool of MEGA7 software and nucleotide changes were clearly observed which indicates the intra specific and inter specific variation. In Fig 8, it has shown the highest sequence similarity between *Thrips florum* and *Thrips hawaiiensis* and showed difference only in the position of guanine in *T. hawaiiensis* in case of adenine in *T. florum*. Barcode gap analysis has shown the presence of cytosine in *T. palmi* but thymine in *Ayyaria chaetophora*.

5.2.5. Phylogenetic analysis

Ananthakrishnan *et al*. (1966) in earlier classified *T. hawaiiensis* under *T. florum* because of the confusing taxonomic characters shared by both species. The phylogenetic tree constructed by using MEGA7 software (Maximum parsimony model) for COI sequences of thrips species in this study revealed that they are closely related species.

5.2.6. Pairwise distance calculation

The inter specific distance between species were computed using K2P (Kimura 2 parameter) from MEGA7 software. The “Distance|Compute Pair-wise” command created the pair wise distances.

In the present study, the range of distance within *T. florum* was 0% to 0.005%. Distance between *T. florum* and *T. hawaiiensis* were 0.003% to 0.005% which indicates their close relationship in phylogeny. Distance between *Ayyaria chaetophora* and *T.palmi* were 0.259% to 0.267%.

5.2.7. Utilization of the developed barcodes in thrips identification

Specimen data comprising taxonomy, specimen images, sequence information and trace files were deposited into BOLD database. Each specimen received distinct process IDs and distinct barcodes were generated. Specific barcodes for each species could be used for the species level allocation of thrips.

Sequences of each specimen submitted to NCBI and generated individual accession numbers which could help the identification of species easily.

6. SUMMARY

The study named “DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala” was carried out at Regional Agricultural Research Station, Pattambi and Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University during the period 2019-2020.The primary objective of the study was to generate DNA barcodes for different species of thrips infesting selected vegetable crops of Kerala and to find out the variability among them. Molecular marker COI locus was used for the study. A total of 21 thrips specimens were collected from various locations of Thrissur and Palakkad districts of Kerala from the vegetable crops in field and greenhouse; cucumber, coccinia, chilli, cowpea, brinjal and tomato. In Thrissur district, *Thrips florum*, *Thrips palmi* and *Ayyaria chaetophora* were obtained. Other than these species *Thrips hawaiienesis* were collected from Koranchira of Palakkad district. *T. florum* were mostly seen in chilli crops under the leaves and inside flowers. *Ayyaria chaetophora* were collected from cowpea, coccinia and brinjal. *T. palmi* were the dominant species in brinjal and tomato grown in greenhouse crops *T. hawaiiensis* was highly identical to *T. florum* were collected from coccinia.

Total genomic DNA extraction of collected specimens were done using Qiagen DNeasy Blood and Tissue Kit and the protocol were standardized. The quality and quantity of extracted DNA were analysed using spectrophotometric method. The absorbance ratio varied from 1.8 to 1.9 which indicated good quality DNA and were appropriate for PCR amplification. Mitochondrial cytochrome oxidase I (COI) gene was used as the barcoding primer for the study. PCR amplification were confirmed by 2% agarose gel electrophoresis (AGE) and generated expected amplicons in the size range of 890 bp. The sequencing was carried out at AgriGenome Lab. Pvt. Ltd., Cochin.

Sequence homology analysis were done using BLASTn tool of NCBI has resulted in a query coverage of 97% to 99% with the identified species. COI sequences containing internal stop codons were detected and trimmed using BioEdit software to make the sequences free from stop codons. Barcode Gap Analysis helps

to anticipate the distribution of distances within each species and the distance to the nearest neighbour of each species. Sequences were readily aligned using clustalW tool of MEGA7 software to identify the similarity in nitrogen bases of the sequences. Multiple sequence alignment revealed highest similarity between *Thrips florum* and *Thrips hawaiiensis* and showed differences in purines which was guanine in *T. hawaiiensis* in case of adenine in *T. florum*. Barcode gap analysis has shown differences in pyrimidine bases which is cytosine in *T.palmi* but thymine in *Ayyaria chaetophora*. Data regarding specimens including taxonomy, specimen images, sequence information and trace files were deposited into BOLD database. Specific process IDs and barcodes were generated by BOLD for each specimen. Inter specific distance between species were computed using K2P (Kimura 2 parameter) from MEGA7 software. The "Distance|Compute Pair-wise" command created the pair wise distances and the range of distance within *T. florum* was 0% to 0.005%. Distance between *T. florum* and *T. hawaiiensis* was 0.003% to 0.005%. Distance between *Ayyaria chaetophora* and *T.palmi* was 0.259% to 0.267%. The phylogenetic tree constructed by using MEGA7 software (Maximum parsimony model) for COI sequences of thrips species has clearly shown that *T. hawaiienesis* and *T. florum* are closely related.

Specific barcodes generated by BOLD for each thrips species could be used for the species level allocation of thrips. Sequences of each specimen submitted to NCBI and generated individual accession numbers which could help the identification of species more conveniently. The precise identification of thrips is the fundamental step to develop genetic and other biological information that is essential for effective management and quarantine measures.

7.REFERENCE

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. 1990. Basic local alignment search tool. [on-line]. Available: https://www.blast.ncbi.nlm.nih.gov. [3 Jan. 2020].

Ananthakrishnan, T.N. and A. Jagaddish. 1966. Studies on some species of the genus Thrips Linn. from India. Entomologisk Tidskrift. 87(2): 85-99.

Barr, N.B., Cui, L. and Mcpheron, B.A. 2005. Molecular systematics of nuclear gene period in genus Anastrepha (Tephritidae). *Ann. Entomol. Soc. Am*. *98*(2): 173-180.

Barrett, J.C., Fry, B., Maller, J.D.M.J. and Daly, M.J. 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. *21*(2): 263-265.

Barrett, R.D. and Hoekstra, H.E. 2011. Molecular spandrels: tests of adaptation at the genetic level. *Nat. Rev. Genet.* *12*(11): 767-780.

Beckingham, K. and Thompson, N. 1982. Under-replication of intron+ rDNA cistrons in polyploid nurse cell nuclei of Calliphora erythrocephala. *Chromosoma*. *87*(2): 177-196.

Bournier, A., A. Lacasa. and Y. Pivot. 1979. Régime alimentaire d'un thrips prédateur *Aeolothrips intermedius* [Thys.: Aeolothripidae]. *Entomophaga*. 24: 353–361.

Brunner, P.C., Fleming, C. and Frey, J.E. 2002. A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) using direct sequencing and a PCR-RFLP-based approach. *Agric. For. Entomol*. *4*(2): 127-136.

Buckman, R.S., Mound, L.A. and Whiting, M.F. 2013. Phylogeny of thrips (Insecta: Thysanoptera) based on five molecular loci. *Syst. Entomol*. *38*(1): 123-133.

Chakraborty, R., Singha, D., Kumar, V., Pakrashi, A., Kundu, S., Chandra, K., Patnaik, S. and Tyagi, K. 2019. DNA barcoding of selected Scirtothrips species (Thysanoptera) from India. Mitochondrial DNA Part B, 4(2): 2710-2714.

Chisholm, I. F. 1983. Feeding mechanism, behaviour and effects of *Limothrips* spp. on cereals. Ph.D. dissertation, University of London, London.

Choi, J.H., Jung, H.Y., Kim, H.S. and Cho, H.G. 2000. PhyloDraw: a phylogenetic tree drawing system. *Bioinformatics*. *16*(11): 1056-1058.

Crespi, B. and Worobey, M. 1998. Comparative analysis of gall morphology in Australian gall thrips: the evolution of extended phenotypes. *Evol*. *52*(6): 1686-1696.

Doull, K. M. 1956. Thrips infesting cocksfoot in New Zealand. II. The biology and economic importance of the cocksfoot thrips *Chirothrips manicatus* Haliday. *N. Z. J. Sci. Tech*. 38: 56–65.

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.

França, L.T., Carrilho, E. and Kist, T.B. 2002. A review of DNA sequencing techniques. Q. Rev. Biophys. *35*(2): 169-200.

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. Resources*. *10*(1): 51-59.

Grinfeld, E. K. 1959. [The feeding of thrips (Thysanoptera) on pollen of flowers and the origin of asymmetry in their mouthparts.] (In Russian). *Ent. Obozr*. 38: 798–804.

Hebert, P.D. and Gregory, T.R. 2005. The promise of DNA barcoding for taxonomy. *Systema. biol*. *54*(5): 852-859.

Heming, B.S. 1978. Structure and function of the mouthparts in larvae of Haplothrips verbasci (Osborn)(Thysanoptera, Tubulifera, Phlaeothripidae). *J. morphol*. *156*(1): 1-37.

Heming, B.S. 1993. Structure, function, ontogeny, and evolution of feeding in thrips (Thysanoptera). *Functional Morphology of Insect Feeding*. 3-41p.

Houston, K.J., Mound, L.A. and Palmer, J.M. 1991. Two pest thrips (Thysanoptera) new to Australia, with notes on the distribution and structural variation of other species. Aust. J. Entomol. *30*(3): 231-232.

Johnson, M.W. 1986. Population trends of a newly introduced species, Thrips palmi (Thysanoptera: Thripidae), on commercial watermelon plantings in Hawaii. *J. Econ. Entomol*. *79*(3): 718-720.

Kadirvel, P., Srinivasan, R., Hsu, Y.C., Su, F.C. and De La Pena, R. 2013. Application of cytochrome oxidase I sequences for phylogenetic analysis and identification of thrips species occurring on vegetable crops. *J. Econ. Entomol*. 106(1): 408-418.

Kurtzman, C.P. 1994. Molecular taxonomy of the yeasts. *Yeast*. *10*(13): 727-1740.

Lewis, T. 1973. *Thrips, their biology, ecology and economic importance.* Academic Press, London.

Macharia, I., Backhouse, D., Skilton, R., Ateka, E., Wu, S.B., Njahira, M., Maina, S. and Harvey, J. 2015. Diversity of thrips species and vectors of tomato spotted wilt virus in tomato production systems in Kenya. *J. Econ. Entomol*. *108*(1): 20-28.

Matsubayashi, K.W., Ohshima, I. and Nosil, P. 2010. Ecological speciation in phytophagous insects. *Entomologia experimentalis et applicata*, *134*(1): 1-27.

Maxam, A. M. & Gilbert, W. 1977. A new method for sequencing DNA. *Proc. natn. Acad. Sci.* 74: 560–564.

Maxam, A. M. & Gilbert, W. 1980. Sequencing end labeled DNA with base-specific chemical cleavages. *Meth. Enzym*. 65: 499–560.

Morgan, A.C. 1913. New genera and species of Thysanoptera, with notes on distribution and food plants. *Proceedings of the United States national Museum.* 46: 1–55.

Moritz, G. 1994. Pictorial key to the economically important species of Thysanoptera in central Europe. *EPPO Bull*. 24:181–208.

Moritz, G., Delker, C., Paulsen, M., Mound, LA. and Burgermeister, W. 2000. Modern methods for identification of Thysanoptera. *EPPO Bull*. 30: 591–593.

Morse, J.G. and Hoddle, M.S. 2006. Invasion biology of thrips. Annu. *Rev. Entomol. 51*:67-89.

Mound L.A. 2004. Thysanoptera: diversity and interactions. *Annu. Rev. Entomol*. 50: 247- 69.

Mound LA, Morris DC. 2003. The morphological background to Thysanoptera phylogeny. *Entomol. Abh*. 61: 151–53.

Mound, L.A. 2002. Thrips and their host plants: new Australian records (Thysanoptera: Terebrantia). *Aust. Entomol*. *29*(2): 49.

Mound, L.A. 2005. Thysanoptera: diversity and interactions. *Annu. Rev. Entomol.*, *50*, pp.247-269.

Murai, T. and Toda, S. 2001. Variation of Thrips tabaci in colour and size, pp. 377Ð378. In: Marullo, R. and Mound, L. (eds), *Thrips and Tospoviruses.* Proceedings of the 7th International Symposium on Thysanoptera, Reggio Calabria, Italy, 377p.

Nakahara, S. 1985. Review of Thrips hawaiiensis and revalidation of T. florum (Thysanoptera:Thripidae). *Proc. Entomol. Soc*. Washington. 87: 864-870.

Pappu, H.R., Jones, R.A.C. and Jain, R.K. 2009. Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. *Virus. Res.141*(2): 219-236.

Ratnasingham, S. and Hebert, P.D. 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PloS one*. *8*(7): 66213.

Rosenheim, J.A., Welter, S.C., Johnson, M.W., Mau, R.F. and Gusukuma-Minuto, L.R. 1990. Direct feeding damage on cucumber by mixed-species infestations of Thrips palmi and Frankliniella occidentalis (Thysanoptera: Thripidae). *J. econ. Entomol*. *83*(4): 1519-1525.

Rubinoff, D., Cameron, S. and Will, K. 2006. A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *J. Hered*. *97*(6): 581-594.

Rugman-Jones, P.F., Hoddle, M.S. and Stouthamer, R. 2010. Nuclear-mitochondrial barcoding exposes the global pest western flower thrips (Thysanoptera: Thripidae) as two sympatric cryptic species in its native California. *J. Econ. Entomol*. *103*(3): 877-886.

Rugman-Jones, P.F., Hoddle, M.S., Mound, L.A. and Stouthamer, R. 2006. Molecular identification key for pest species of Scirtothrips (Thysanoptera: Thripidae). *J. Econ. Entomol*. *99*(5): 1813-1819.

Saccone, C., De Giorgi, C., Gissi, C., Pesole, G. and Reyes, A. 1999. Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. *Gene*. *238*(1): 195-209.

Sakimura, K., Nakahara, L.M. and Denmark, H.A. 1986. A thrips, Thrips palmi Karny. Florida Dept. *Agric. & Consumer Serv., Div. Plant Ind. Entomol. Circ*, (280).

Sanger, F. & Coulson, A. R. 1975. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J. Mol. Biol.* 94: 441–448

Saraste, M. 1990. Structural features of cytochrome oxidase. *Q. Rev. Biophys*. *23*(4). 331-366.

Seal, D.R. and Klassen, W. 2005. Chilli thrips (castor thrips, Assam thrips, yellow tea thrips, strawberry thrips) *Scirtothrips dorsalis Hood*, Provisional Management Guidelines. *Document ENY-725 University of Florida, Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences*.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. and Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am*. 87(6): 651-701.

Tyagi, K., Mound, L. and Kumar, V. 2008. Sexual dimorphism among Thysanoptera Terebrantia, with a new species from Malaysia and remarkable species from India in Aeolothripidae and Thripidae. *Insect. Syst. Evol.* *39*(2): 155-170.

Tyagi, K., Kumar, V., Singha, D., Chandra, K., Laskar, B.A., Kundu, S., Chakraborty, R. and Chatterjee, S. 2017. DNA Barcoding studies on Thrips in India: Cryptic species and Species complexes. *Scientific reports*. *7*(1): 1-14.

Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol*. *3*(5): 294-9.

Walsh BJ. 1867. The apple-worm and the apple maggot. J. Hortic. 2: 338–343.

Whitfield, A.E., Ullman, D.E. and German, T.L. 2005. Tospovirus-thrips interactions. *Annu. Rev. Phytopathol.*, *43*(8): 459-489.

Yang, Z. 2007. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol*. *24*(8): 1586-1591.

Yeh, W.B., Tseng, M.J., Chang, N.T., Wu, S.Y. and Tsai, Y.S. 2015. Agronomically important thrips: development of species-specific primers in multiplex PCR and microarray assay using internal transcribed spacer 1 (ITS1) sequences for identification. *B. Entomol. Res*. *105*(1): 52-59.

Zhang, D.X. and Hewitt, G.M. 1997. Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies. *Biochem. Syst. Ecol*. *25*(2): 99-120.

Zhou, X., Ren, L., Li, Y., Zhang, M., Yu, Y., and Yu, J. 2010. The next-generation sequencing technology: a technology review and future perspective. *Sci. China Life Sci.* 53(1): 44-57.

ANNEXURE I

Materials used during collection of thrips specimen

- Polythene bags
- Rubber bands
- Microscope: 10X,30X and 40X lens
- Needle
- Paint brush (Zero size)
- 2ml vials
- 70% ethanol

ANNEXURE II

Reagents used for DNA isolation

- **QIAGEN DNeasy Blood and Tissue Kit**

  - ATL Buffer
  - Proteinase K
  - AL Buffer
  - 100% Ethanol
  - AW1 Buffer
  - AW2 Buffer
  - AE buffer

ANNEXURE III

Composition of buffers and dyes used for agarose gel electrophoresis (AGE)

1. TAE Buffer 50X

Tris base: 242 g

Glacial acetic acid: 57.1ml

0.5 M 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 10 mg/ml; working concentration 0.5 µg/ml (SRL) and was stored at room temperature in a dark bottle.

4. **Agarose** - 0.8 per cent (Genomic DNA)- 1.5 per cent (for PCR samples).

5. **Electrophoresis unit**- Bio-Rad power PAC 1000, gel casting tray, comb.

List of laboratory equipment's used for the study

Centrifuge : REMI C-24BL

Waterbath : ROTEK

Microwave oven: LG

-20 Refrigerator: Labline

Gel documentation system: **GELSTAN 4x Advanced**

Thermal cycler: Eppendorf

Spectrophotometer: Systronics

ABSTRACT

The study entitled “DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala” was carried out at Regional Agricultural Research Station, Pattambi and Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University during the period 2019-2020. The primary objective of the study was to generate DNA barcodes for different species of thrips infesting selected vegetable crops of Kerala and to find out the variability among them.

The family Thripidae insects are also known as thrips, a tiny sewing needle like complex plant pest in the size of 0.5-1.5 millimetres in length. Morphological identification of thrips species is very difficult because of their minute size, scarcity of solid morphological characters, high cryptic behaviour, sexual dimorphisms and overlapping host ranges and the need for taxonomic expertise. DNA barcoding is the most desirable tool to identify known and unknown species and phylogenetic analysis based on the nucleotide arrangement of sequence in DNA fragment by the use of a short-standardized DNA sequence of 658 bp fragment of the mitochondrial cytochrome cytochrome oxidase (COX I) gene.

A total of 21 thrips specimens were collected from various locations of Thrissur and Palakkad districts of Kerala from vegetable crops like cucumber, coccinia, chilli, cowpea, brinjal and tomato. Morphological identification was done by Zoological Survey of India and identified four species which are *Thrips florum, Thrips palmi, Thrips hawaiiensis and Ayyaria chaetophora.* Total genomic DNA extraction of collected specimens were done using Qiagen DNeasy Blood and Tissue Kit. The extracted DNA was used to amplify the cytochrome oxidase subunit 1 gene, using the selected primers and gave amplicons in the size range 890 bp. The sequencing process was carried out at AgriGenome Lab. Pvt. Ltd., Cochin. Sequence homology analysis were done with the help of BLASTn tool of NCBI has resulted in a query coverage of 97% to 99% with the identified species. Barcode Gap Analysis helps to anticipate the distribution of distances within each species and the distance

to the nearest neighbour of each species. Sequences were readily aligned using clustalW tool of MEGA7 software and the similarity in nitrogen bases of the sequences were identified. Multiple sequence alignment revealed highest similarity sequences between *Thrips florum* and *Thrips hawaiiensis* and showed difference only in the position of guanine in *T. hawaiiensis* in case of adenine in *T. florum*. Barcode gap analysis has shown the presence of cytosine in *T.palmi* but thymine in *Ayyaria chaetophora*. Data regarding specimens including taxonomy, specimen images, sequence information and trace files were deposited into BOLD database. Specific process IDs and barcodes were generated by BOLD for each specimen. The inter specific distance between species were computed using K2P (Kimura 2 parameter) from MEGA7 software. The "Distance|Compute Pair-wise" command created the pair wise distances. The range of distance within *T. florum* was 0% to 0.005%. Distance between *T. florum* and *T. hawaiiensis* was 0.003% to 0.005%. Distance between *Ayyaria chaetophora* and *T.palmi* was 0.259% to 0.267%. The phylogenetic tree constructed by using MEGA7 software (Maximum parsimony model) for COI sequences of thrips species has clearly shown that *T. hawaiienesis* and *T. florum* are closely related species. DNA sequences of 21 thrips species were deposited in the NCBI GenBank and accession numbers were generated could help the identification of species easily and specific barcodes generated for each species could be used for the species level allocation of thrips. The precise identification of thrips is the fundamental step to develop genetic and other biological information which is essential for effective management and quarantine measures.

"DNA barcoding of thrips (Thysanoptera: Thripidae) associated with selected vegetable crops of Kerala"

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(2015-09-023)

ABSTRACT OF THESIS

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DEPARTMENT OF PLANT BIOTECHNOLOGY

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2020

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