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Genetic diversity and species prevalence revealed by Mitochondrial COI- DNA sequence of thrips population in cotton

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Abstract

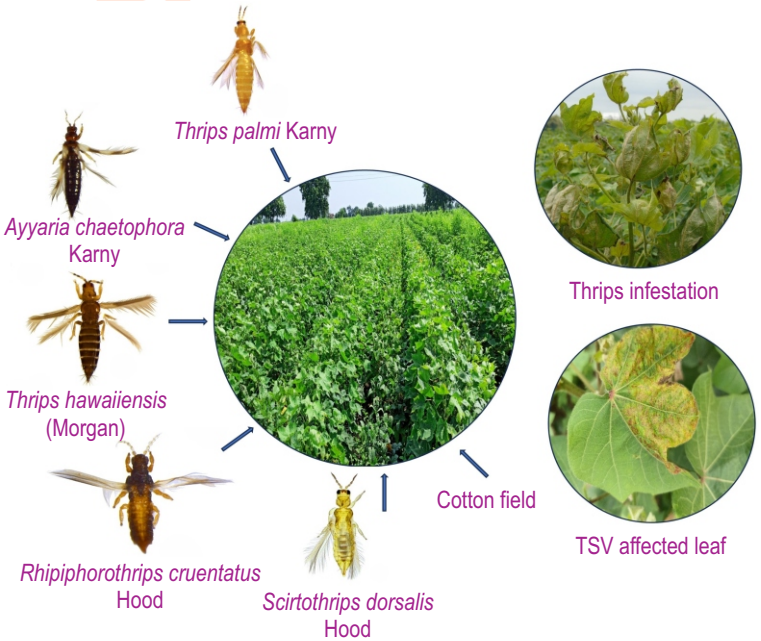
Aim: To elucidate the population genetic diversity and species prevalence of thrips on cotton.

Methodology: Thrips species were identified utilizing both mitochondrial cytochrome oxidase subunit I (COI) sequence analysis and morphology-based keys.

Results: *Thrips palmi* Karny, *Thrips hawaiiensis* (Morgan), *Ayyaria chaetophora* Karny, *Scirtothrips dorsalis* Hood and *Rhipiphorothrips cruentatus* Hood identified from collected samples. The distribution analysis revealed that *T. palmi* stands out as the most abundant and prevalent species, constituting a substantial majority (74%) and occupying a pervasive presence across most cotton-growing locations. The phylogenetic analysis, relying on COI sequences, clearly categorized these five species into four distinct major clades. *T. hawaiiensis* and *R. cruentatus* were placed within the first clade, while the second clade featured *Scirtothrips dorsalis.* The third clade comprised the abundant *T. palmi,* and the fourth clade accommodated *A. chaetophora*.

Interpretation: The study highlighted genetic diversity of five species of thrips, accentuating the emergence of *T. palmi* as a dominant pest in cotton, an observation not previously reported.

Key words: Cotton thrips, Genetic diversity, *MtCOI*, Phylogenetic analysis, Species prevalence



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Introduction

Thrips, belonging to the order Thysanoptera, are tiny insects with fringe wings, ranging in size from 0.2 to 15 mm, and are found worldwide. The global thrips inventory encompasses 7,700 identified species, classified into two suborders: Terebrantia, characterized by a blunt posterior end, and Tubulifera, distinguished by a tube-like abdominal end. This classification further extends to nine families and five subfamilies (Moritz, 1994; Moritz *et al.,* 2000). Within the diverse spectrum of thrips, India is home to 739 identified species (Tyagi and Kumar, 2016). Remarkably, 180 of these species have been recognized as significant sucking pests, inflicting substantial yield losses through direct feeding on agricultural and horticultural crops (Mound and Palmer, 1981). Moreover, 15 species have assumed special attention globally for their exclusive role as transmitters of tospoviruses, a distinction that has drawn extensive research focus (Pappu *et al.,* 2009; Riley *et al.,* 2011; Stuart *et al.,* 2011; Zhou and Tzanetakis, 2013; Mouden *et al.,* 2017) and two species transmitting *Pantoea* spp. causing center rot of onion (Gitaitis *et al.,* 2003; Dutta *et al.,* 2014).

In addition to their pestiferous role, certain thrips species exhibit a more benign dietary preference, feeding on fungi, dead leaves, and branches. These thrips serve as potential pollinators or biological control agents, contributing significantly to nutrient recycling (Mound and Kibby, 1998; Trdan *et al.,* 2005). This dual role highlights the ecological complexity of thrips, emphasizing their importance not only as pests but also as contributors to ecosystem dynamics and balance. In the Indian context, cotton, the primary fibre crop, occupy the largest cultivated area and ranks second globally in terms of production. This vital crop faces a challenge from a spectrum of 251 arthropod pests in India (Nagrare *et al.,* 2022); with thrips emerging as a prominent sucking pest that inflicts damage from the seedling stage to the boll opening stage. Both larvae and adults of thrips scraps developing leaves, flowers, and bolls, extracting sap and causing direct harm, displaying as discoloration, deformities, and a subsequent reduction in plant vigour. Thrips wreak havoc on cotton seedlings by rasping tender leaves and terminal buds with their sharp mouthparts, resulting in browning, silvering, and distortion of leaf edges.

Severe infestations lead to upward leaf curling, while even milder cases hamper plant growth and hinder crop maturity. Beside direct damage, thrips also play the role of vector of Tobacco Streak Virus (TSV) in cotton (Kumar *et al.,* 2017; Rageshwari *et al.,* 2017, Muppudathi *et al.,* 2020) and center rot of onion (*Allium cepa* L.) caused by bacterial pathogens, *Pantoea ananatis* and *P. agglomerans,* transmitted by onion thrips (*Thrips tabaci*) (Dutta *et al.,* 2014) and *P. ananatis* causing center rot of onion transmitted by tobacco thrips (*Frankliniella fusca*) (Gitaitis *et al.,* 2003). Survey and monitoring efforts have revealed an unprecedented surge in thrips populations, particularly in Central and North India over the last 4-5 years. Notably, North India recorded a staggering 94.20 thrips per 3 leaves during the 30th standard meteorological week (23 July to 29 July). Conventional methods such as insecticide application, host plant resistance, and natural enemy augmentation, though core components of thrips management, have witnessed diminished effectiveness in recent times. Importantly, it has been observed that different thrips species respond differently to management methods. Therefore, accurate identification and understanding the composition of thrips species within the cotton ecosystem become paramount prerequisites for formulating effective pest management strategies. This nuanced approach is essential to address the evolving challenges posed by thrips and safeguard the productivity of the cotton crop.

Globally, the cotton crop infested by 16 thrips species, including the tobacco thrips (*Frankliniella fusca* Hinds), flower thrips (*Frankliniella tritici* Fitch), common blossom thrips (*Frankliniella schultzei* Trybom), western flower thrips (*Frankliniella occidentalis* Pergande), *Frankliniella brevicaulis* Hood, *Frankliniella cephalica* Crawford, cotton thrips (*Thrips tabaci* Lindeman), melon thrips (*Thrips palmi* Karny), soybean thrips (*Neohydatothrips variabilis* Beach), Kurtoma thrips (*Kurtomathrips morrilli* Moulton), bean thrips (*Caliothrips fasciatus* Pergande), cotton leaf thrips (*Caliothrips sudanensis* Bagnall), dark cotton leaf thrips (*Caliothrips impurus* Preisner), black vine thrips (*Retithrips syriacus* Mayet), composite thrips (*Microcephalothrips abdominalis* Crawford DL), and chili thrips (*Scirtothrips dorsalis* Hood) (Watts, 1936; Attique and Ahmad, 1990; Bournier, 1994; Leighl, 1995; Atakan *et al.,* 1996; Cook *et al.,* 2003; Albeldaño *et al.,* 2008; Kerns and Anderson, 2012; Stewart *et al.,* 2013; Ebratt-Ravelo *et al.,* 2019). This comprehensive list underscores the global complexity of thrips species affecting cotton, with each species posing distinct challenges and considerations for effective pest management.

The diversity of these thrips species reflects the ongoing efforts to understand and address the complex dynamics of thrips infestations in cotton cultivation worldwide. In the Indian context, about nine thrips species have been reported to infest cotton. These include *Asprothrips bimaculatus* Michel & Ryckewaert, orchid thrips (*Chaetanaphothrips orchidii* Chanor), common blossom thrips (*Frankliniella schultzei* Trybom), chili thrips (*Scirtothrips dorsalis* Hood), banana flower thrips (*Thrips florum* Schmutz), Hawaiian flower thrips (*Thrips hawaiiensis* Morgan), melon thrips (*Thrips palmi* Karny), Taiwanese thrips (*Thrips parvispinus* Karny), and cotton thrips (*Thrips tabaci* Lindeman) (Mound and Palmer, 1981; Ananthakrishnan, 1984; Padaliya *et al.,* 2019; Gupta *et al.,* 1997; Kaur *et al.,* 2021; Amutha and Rachna, 2023). This compilation reveals the ongoing efforts to identify and document the diverse thrips species associated with cotton in India, providing a basis for targeted pest management strategies in the region. Certain thrips species exhibit a complex and cryptic nature, posing challenges to traditional identification methods (Rugman-Jones *et al.,* 2010). The minute size of thrips, coupled with morphological variations, intraspecific polymorphisms, sexual dimorphisms, and the time-intensive nature of traditional taxonomic keys, complicates their accurate

identification (Brunner *et al.,* 2002; Tyagi *et al.,* 2008; Sartiami and Mound, 2013). Fortunately, molecular techniques provide a modernized solution, offering a simplified and more accurate means of identifying various insect species, including thrips. By overcoming the challenges associated with morphological diversity across life stages and minute morphological distinctions between species, molecular techniques enhance the precision and expediency of thrips diagnosis (Brunner *et al.,* 2002; 2004).

At present variety of molecular markers are available for characterizing the genetic diversity of insects (Reddy *et al.,* 1999; Sreejith and Sebastian, 2015). In molecular identification, the utilization of mitochondrial DNA markers, particularly for phylogenetic studies in the animal kingdom, proves invaluable in distinguishing closely related species. Therefore, these markers have gained popularity for critical genetic variations within individuals, as well as across related species and populations (Mandal *et al.,* 2014). The mitochondrial cytochrome oxidase I gene (COI) emerge as a widely employed and universal molecular marker for insect identification (Hebert *et al.,* 2003). Notably, the mitochondrial genes of thrips exhibit a relatively fast evolutionary rate (Shao and Baker, 2003). The application of the COI 5' region in tests designed to unveil cryptic insect species, including thrips, has been well established (Hebert *et al.,* 2004; Glover *et al.,* 2010; Rebijith *et al.,* 2014; Iftikhar *et al.,* 2016; Tyagi *et al.,* 2017). This emphasises the efficacy of molecular tools, specifically the COI gene, in unravelling complicated genetic distinctions within the diverse domain of thrips and other insect species. Genomic DNA-based species discrimination approach is highly reliable and can also be employed to resolve a species ambiguity which is often not possible with conventional taxonomy. Destructive behaviour as a sucking pest and the varying potential of thrips to transmit viral diseases (Ananthakrishnan and Annadurai, 2007) and bacterial diseases (Dutta *et al.,* 2014; Gitaitis *et al.,* 2003) provides an additional incentive to explore their genetic diversity. Similarly, to resolve the taxonomic issues of identification, molecular-based identification is a primary step for precise identification of species and to develop effective management strategies. With this view, the present investigation was taken up to elucidate the population genetic diversity and prevalence of thrips species in cotton ecosystem.

Materials and Methods

Thrips sample collection: To ascertain the species composition and genetic diversity among the thrips in cotton ecosystem, surveys were undertaken once per location to collect the thrips population during vegetative stage of crop when the infestation was at its peak in different major cotton growing districts of India. Total of 353 adult thrips samples were collected from 23 locations comprising 15 districts from 5 major cotton-growing states of India (Fig. 1). The samples were collected by beating methods (Powelll and Landis, 1965) in a white plastic tray. The collected samples from different field locations were preserved in 70% molecular grade ethanol (HIMEDIA, MB106) by labelling it according to locations and stored at -20°C for further investigations. These samples were further used for morphological examination and molecular studies.

Morphological analysis: To study morphological features, five insect specimens were taken from each location from uniform population of collected samples. The specimens were mounted on a slide (Mound and Kibby, 1998) and the photographs were taken through a Leica binocular Microscope (Leica M205A) using a Leica software application suite (LAS V4.12). The specimens were then compared with standard taxonomic keys of each identified species (Goldarazena, 2011; Zhang *et al.,* 2011; Natasa and Stanislav, 2012; Sartiami and Mound, 2013; IPPC, 2016; Cluever and Smith, 2017). Further, the specimens were also confirmed from the Zoological Survey of India (ZSI), Kolkata and National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bangaluru, India. Based on morphological similarity, the representative a single thrips was used for further molecular characterization.

Molecular characterization and analysis

Genomic DNA extraction: Genomic DNA from individual thrips was extracted using slightly modified protocol developed by Frohlich *et al.* (1999) for genomic DNA extraction of whitefly. The thrips was ground in 40 µl ice-cold tissue lysis buffer (containing, 5 µM Tris HCl, pH 8.0, 0.5 µM EDTA, 0.5% Nonidet P-40 and 1 mg/ml proteinase K) and then incubated at 65°C for 20 min followed by an incubation at 95°C for 10 min to inactivate the proteinase K. The lysate was then centrifuged at 10000 rpm for 10 min. The quantity and quality of the isolated DNA were determined using Nanodrop Spectrophotometer (Implen, NP80-Touch) and 1.2% agarose gel stained with ethidium bromide. The resultant aqueous supernatant was used as a DNA template for PCR amplification using the universal COI primer pair: C1-J-1751:F-5'-GGATCACCTGATATAGCATTCCC-3'(CO-I 3F) and C1-N-2191:R-5'-CCCGGTAAAATTAAAATATAAACTTC-3'(CO-I 3R) (Simon *et al.,* 1994; Fekrat *et al.,* 2015). These primers were expected to yield an amplicon of approximately 439 bp using an Eppendorf Master cycler®.

PCR amplification of a COI fragment and Sequencing: A 25 µl PCR reaction mixture contained 1.00 µl DNA template, 0.4 µl dNTPs, 4 µl 5X buffer 1.4 µl $MgCl_2$, 0.3 µl Taq polymerase, 0.5 µM 16.9 µl nuclease free water, and 0.5 µl of each forward and reverse primers. The PCR amplification procedure was followed as: Initial denaturation -94°C for 4 min, for 35 cycles of 94°C for 45 sec, 51°C for 45 sec annealing, 72°C for 1 min and a final extension at 72°C for 7 min. The resultant PCR products were examined by 1.5% agarose gel electrophoresis. Further, the amplified PCR products were directly sent to Agri Genome Lab Pvt. Ltd., Kochi (India) for Sanger sequencing. The consensus sequences of the partially assembled sequences were done using CAP3: A DNA sequence assembly program (Huang and Madam, 1999). These assembled sequences were subjected to run in ExPASy Translate tool (https://web.expasy.org/translate/) to allow the translation of nucleotide (DNA) sequence to a protein

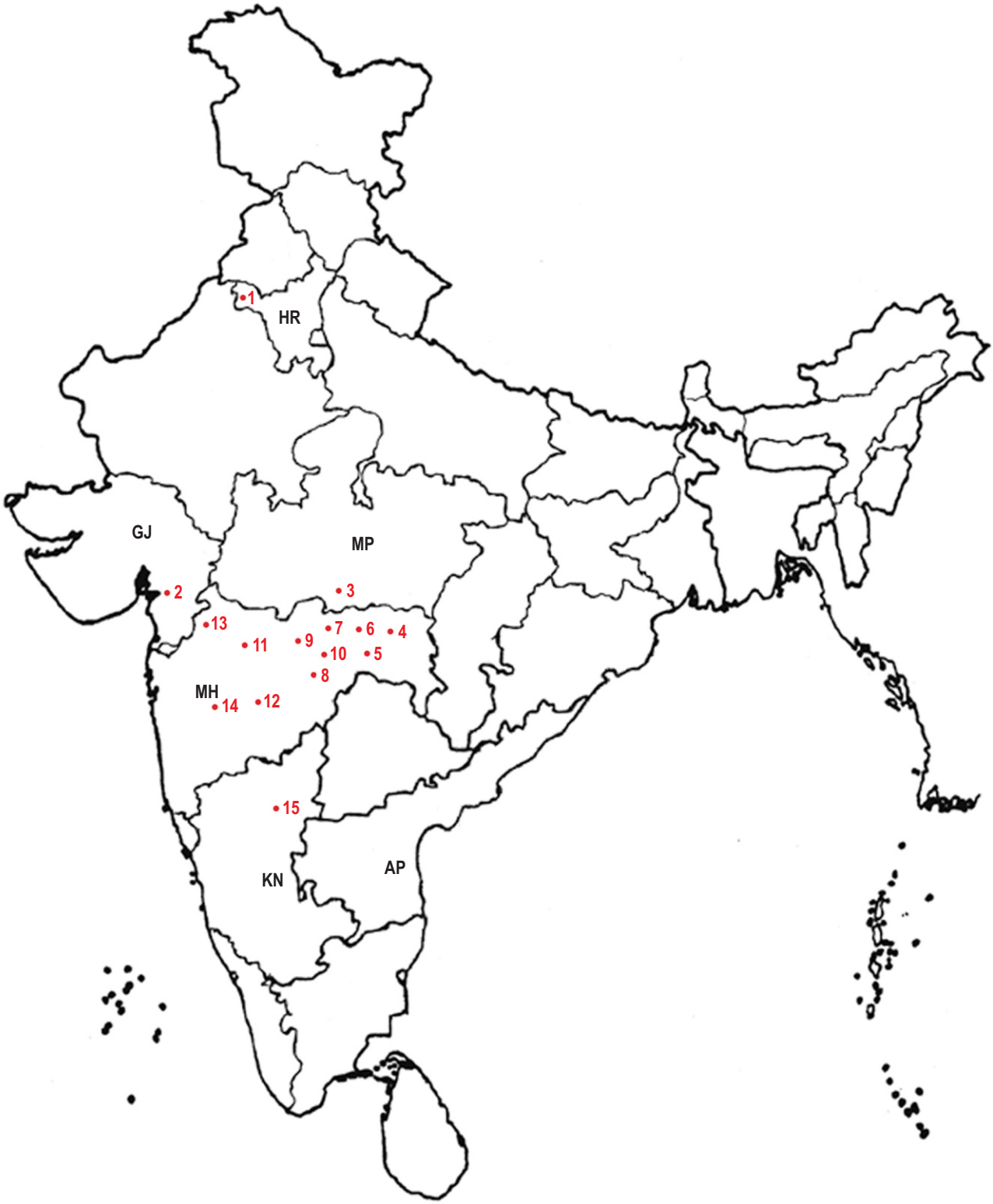

Fig. 1: Map of India showing the location of thrips sample collection. (1) Haryana (HR): Sirsa; (2) Gujarat (GJ): Bharuch; (3) Madhya Pradesh (MP): Chhindwada; (4) Maharashtra (MH): Nagpur; (5) Wardha; (6) Amravati; (7) Akola; (8) Yavatmal; (9) Buldhana; (10) Washim; (11) Jalgaon; (12) Parbhani; (13) Dhule; (14) Ahemadnagar; (15) Karnataka (KN): Raichur.

sequence which was further submitted to NCBI GenBank database through BankIt.

Phylogenetic analysis: Phylogenetic analysis was performed in IQtree 1.6.12 (Hoang *et al.,* 2018) using the SH-aLRT (SH-like approximate likelihood ration test) and UFBoot (Ultrafast Bootstrap) method with 10,000 replicates involving 28 sequences. The sequences of *T. tabaci* were retrieved from NCBI database while all other sequences used were yielded from the present investigation (Fig. 3).

Results and Discussion

Morphological identification: Morphological studies and identification of samples from twenty-three locations confirm 5 species from 4 genera viz. *Thrips* Linnaeus, *Rhipiphorothrips* Morgan, *Scirtothrips* and Ayyaria Karny belongs to family Thripidae. Species identified as *Thrips palmi* Karny had entirely yellow-coloured body, 1st and 2nd antennal segments and yellow 3rd segment with brownish shedding on the apex portion. Metanotal sculpture striate; lacking microtrichia on lateral thirds of tergites IV–VI. As the antennal segments shows increase in colour, the antenna becomes yellowish to brownish gradually from base to apex. Red-coloured triangle was prominent on the top of the head. Pores present on mesonotum; merely two pairs of long coarse hairs on pronotum. *Ayyaria chaetophora* Karny was identified by the most prominent character, the banded pattern of fore wings (white with two dark crossbands), elongated body and 8 segmented antennae. Species identified as *Rhipiphorothrips cruentatus* Hood possesses brown body, antennae and legs clearly yellow, forewings with pale yellow veins, and rugose sculpture on the head. *Thrips hawaiiensis* (Morgan) having prominent characters as brown abdomen, yellow to orange brown thorax and head, yellow to yellowish brown legs, postocular setae II subequal to III, abdominal sternites with 10-25 discal setae, antennae brown with 7-8 segments. Forewings clavus with apical setae elongated than subapical setae. In *Scirtothrips dorsalis* both sexes are fully winged. Body mainly yellow, antennal segment I white, II & III pale, IV-VIII brown, major setae not dark. Metanotal median area with anterior transverse sculpturing, and posterior half with irregular longitudinal reticulations, median setae arise behind anterior margin, campaniform sensilla absent. Tergites III-VII with small median setae, II-VIII laterally covered in regular rows of fine microtrichia. Thus, morphological studies facilitated successful identification of all the samples in five major thrips species, *T. palmi, T. hawaiiensis, R. cruentetus, S. dorsalis,* and *A. chaetophora* collected from the cotton ecosystem.

Molecular identification: PCR amplification using the universal COI primer, a uniform amplicon size of ~439 bp was observed in all the collected samples from different locations under study. Initially, a homology search was performed using BLAST (www.ncbi.nlm.nih.gov). Based on sequence comparison, all the fragments of COI gene sequences of thrips samples collected from 23 locations were identified and confirmed to 5 species of thrips viz. *T. palmi, T. hawaiiensis, R. cruentatus, S. dorsalis* and *A. chaetophora.* Comparison of the sequences with those in GenBank and BOLD database revealed that 11 samples of *T. palmi* showed 100% sequence similarity matches with the NCBI GenBank database sequences and 6 samples showed 99% similarity. However, *Thrips hawaiiensis, R. cruentatus* and *Scirtothrips dorsalis* showed 100% similarity whereas 3 samples of *A. chaetophora* showed 91% similarity with NCBI nucleotide sequences. The sequences were deposited in the NCBI GenBank database with allotted accession numbers (Table 1). The corresponding distribution of these species in cotton ecosystem was recorded as ~74, ~13, 5, for *T. palmi, A. chaetophora, T. hawaiiensis* and 4% each for *R. cruentatus* and *S. dorsalis* respectively (Fig. 2). Distribution analysis confirmed that presently *T. palmi* is the most abundant and predominant species found infesting cotton.

Species identification results revealed that composite collection of adult cotton thrips samples from locations including Nagpur, Amravati, Akola, Wardha, Warud (Amravati), Yavatmal, Buldana, Jalgaon, Dhule, Ahmadnagar, Parbhani from Maharashtra were identified as *T. palmi.* The same species of thrips was identified from the samples collected from the cotton crop from Pandhurna (MP), Bharuch (Gujarat), Sirsa (Haryana) and Raichur (Karnataka). However, *R. cruentatus* was identified from Katol (Nagpur, Maharashtra) and *S. dorsalis* from Chhindwara (Madhya Pradesh). Similarly, thrips samples collected from the experimental fields of ICAR-CICR, Nagpur and from Parbhani, Maharashtra were identified as *A. chaetophora.* Whereas, the samples from Washim, Maharashtra were identified as *T. hawaiiensis* (Table 1).

The phylogenetic relationships among thrips species were investigated using partial mtCOI sequences, comparing them to known mtCOI sequences of thrips available in the NCBI GenBank database. Sequence alignment was performed using the MEGA 6 software package (Tamura *et al.,* 2013), followed by phylogenetic analysis employing IQtree 1.6.12 software (Hoang *et al.,* 2018) with 10,000 replicates. The resulting tree was visualized in Dendroscope (Huson and Scornavacca, 2012), as shown in Fig. 3. The analysis yielded a well-resolved tree that highlighted the distinctness of several thrips species. Specifically, *T. palmi, A. chaetophora, S. dorsalis were clearly delineated, while R. cruentatus* and *T. hawaiiensis* appeared as sister species. This phylogenetic tree, constructed from mtCOI sequences of thrips samples from 23 locations, revealed four distinct clades gathered from 16 cotton-growing districts across 5 states in India. *Thrips palmi* emerged as the predominant thrips species in the Indian cotton ecosystem, exhibiting 99% sequence similarity. Notably, *S. dorsalis* occupied its own distinct node in clade 2, sharing a common ancestry with *T. palmi.* Within *T. palmi,* seventeen specimens clustered into two sister clades within the main clade 3 (Fig. 3). Additionally, *A. chaetophora* formed a separate, well-supported clade (clade 4) with 100% branch support. Meanwhile, *T. hawaiiensis* and *R. cruentatus* were identified as sister species within their distinct clade (clade 1) in the phylogenetic tree (Fig. 3).

Table 1: Identification results of thrips samples collected from cotton ecosystem

Sample code	Districts	Species identified	Family	Gene bank accession number for COI fragment	% similarity with NCBI submitted sequences
Th -NAG	Nagpur	*Thrips palmi* Karny	Thripidae	MK134705	100
Th-KAT	Katol (Nagpur)	*Rhipiphorothrips cruentatus* Hood	Thripidae	MK192989	100
Th-AMR	Amravati	*Thrips palmi* Karny	Thripidae	MK192984	100
Th-AKL	Akola	*Thrips palmi* Karny	Thripidae	MK192983	100
Th-WAR	Wardha	*Thrips palmi* Karny	Thripidae	MK192986	99
Th-WARU	Warud (Amravati)	*Thrips palmi* Karny	Thripidae	MK192993	100
Th-WAS	Washim	*Thrips hawaiiensis* (Morgan)	Thripidae	MK192985	100
Th-YAV	Yavatmal	*Thrips palmi* Karny	Thripidae	MK192987	100
Th- CICR1	Nagpur	*Ayyaria chaetophora* Karny	Thripidae	MK192998	91
Th- CICR2	Nagpur	*Ayyaria chaetophora* Karny	Thripidae	MK192999	91
Th-BUL	Buldhana	*Thrips palmi* Karny	Thripidae	MK192996	100
Th-JAL	Jalgaon	*Thrips palmi* Karny	Thripidae	MK192995	100
Th-DHU	Dhule	*Thrips palmi* Karny	Thripidae	MK192988	100
Th-PAR	Parbhani	*Thrips palmi* Karney	Thripidae	MK192994	100
Th-PARB	Parbhani	*Ayyaria chaetophora* Karny	Thripidae	MK192997	91
Th- RHA1	Rahuri (Ahmadnagar)	*Thrips palmi* Karny	Thripidae	MK193004	99
Th- RHA2	Rahuri (Ahmadnagar)	*Thrips palmi* Karny	Thripidae	MK193005	99
Th-PAN	Pandhurna	*Thrips palmi* Karny	Thripidae	MK193002	99.67
Th-BHA1	Bharuch	*Thrips palmi* Karny	Thripidae	MK193001	99.15
Th-BHA2	Bharuch	*Thrips palmi* Karny	Thripidae	MK193000	99.15
Th-CHI	Chindwara	*Scirtothrips dorsalis* Hood	Thripidae	MK193003	100
Th-SIR	Sirsa	*Thrips palmi* Karny	Thripidae	MK192991	100
Th-KAR	Raichur	*Thrips palmi* Karny	Thripidae	MK192990	100

Correct identification of thrips species using modern tools holds the key in devising management strategies and its subsequent implementation to prevent it from its surge in crop ecosystem. Cytochrome oxidase (COI) sequencing used in the present study clearly distinguished the thrips populations from cotton ecosystem at species level as reported in previous studies in different crops (Brunner *et al.,* 2002; Brunner and Frey, 2010; Glover *et al.,* 2010; Kadirvel *et al.,* 2013; Yeh *et al.,* 2015). In the present study, morphological and molecular analysis confirmed a complex of five species of thrips in cotton-growing states of India viz. *T. palmi, R. cruentatus, A. chaetophora, S. dorasalis* and *T. hawaiiensis.* However, not a single specimen of *Thrips tabaci* Lindeman, which is often reported as dominant species in cotton (Gupta *et al.,* 1997; Sharma and Pampapathy, 2006; Kaur *et al.,* 2021) was found in the collected samples. Thrips palmi found to be the most abundant while *R. cruentatus, A. chaetophora, S. dorsalis* and *T. hawaiiensis* though recorded at few locations but infest cotton. Muppudathi *et al.* (2020) reported *T. palmi* as the highly prevalent thrips species established in cotton ecosystem in Tamil Nadu, India using morphological (ESEM) and molecular characterization (RT-PCR using mtCOI primers) which are in accordance with present findings. Similarly, comprehensive study on cotton thrips diversity was conducted (Senguttuvan, 2019) in cotton ecosystem of Tamil Nadu based on collection and random surveys. Experimental results of phylogenetic analysis and diversity indices revealed the prevalence of *S. dorsalis* Hood (3.25) followed by *T. tabaci* (1.34) and *T. palmi* (0.24) and concluded existence of high diversity of thrips species occurring in cotton ecosystem system in Tamil Nadu. Senguttuvan, (2019) has also reported *S. dorsalis* as an alarming key pest of cotton in Tamil Nadu. Recently, from DNA barcoding studies of thrips species in India carried out by Tyagi *et al.* (2017) revealed presence of cryptic diversity using Neighbour-Joining (NJ) and Bayesian analysis in the four clades in *T. palmi.* Further, three species, *T. hawaiiensis, T. florum* and *T. andrewsi* under the 'hawaiiensis species complex' formed three separate clades with high support values. These findings are in line with the results of the present study which confirms the presence of cryptic species of thrips in cotton ecosystem. Climate change may also have significant effects on the diversification of thrips species (Garrick and Liburd, 2018). In this context, results obtained from the present study has a great relevance as very few reports showed *T. palmi* as dominant species in Indian cotton ecosystem.

The phylogenetic analysis revealed the selected thrips population's arrangement into four primary clades. *Thrips palmi* stood out as the most abundant, showcasing a 99.9% sequence similarity. Notably, 75% of the samples formed part of clade 3, which featured two sub-clades. Conversely, *A. chaetophora*, represented by three samples, grouped within clade 4. Mean while, *T. hawaiiensis* and *R. cruentatus* clustered together in clade 1, depicting close genetic similarity between them. Clade 2

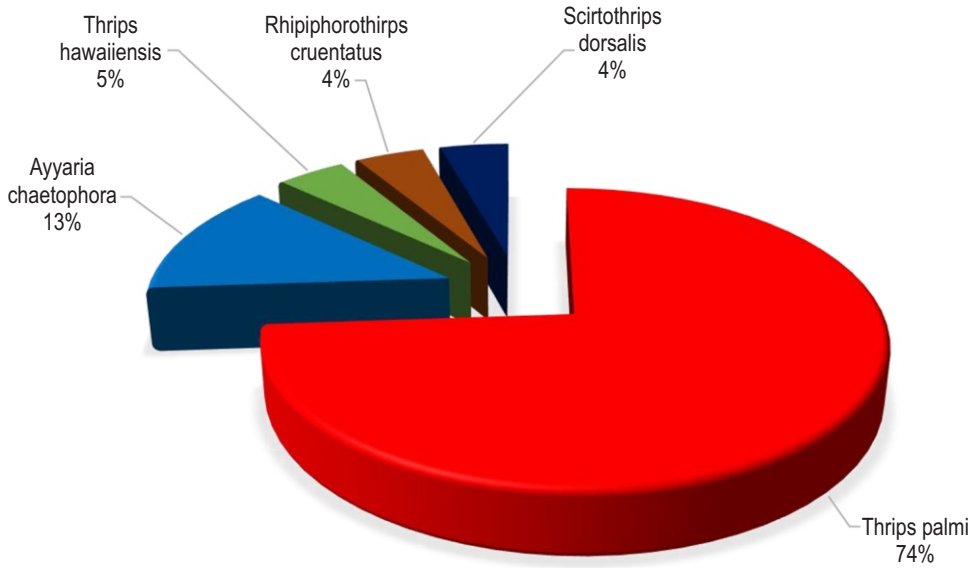

Fig. 2: Distribution pattern of different thrips species in cotton ecosystems.

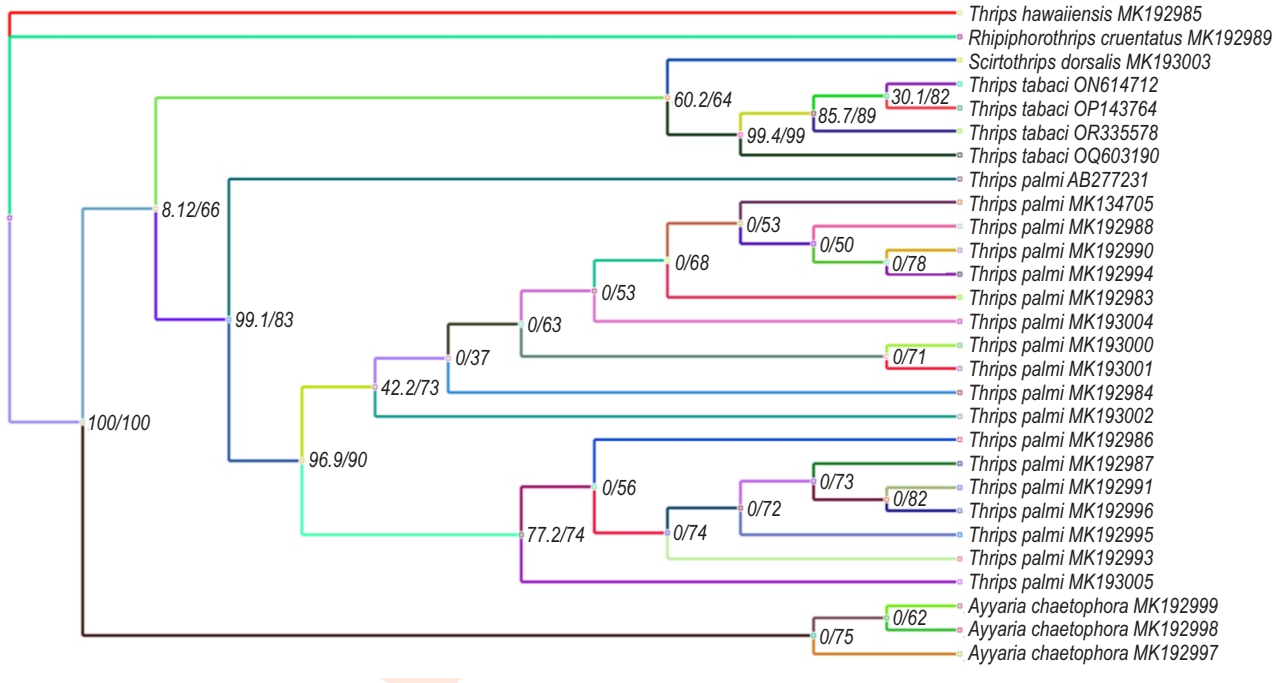

Fig. 3: Dendrogram of phylogenetic relationships amongst the thrips species assessed using SH-aLRT (SH-like approximate likelihood ration test) and UFBoot (Ultrafast Bootstrap) with 10000 replicates.

was characterized by the presence of *Scirtothrips dorsalis*. Similar clades of *T. palmi* in phylogenetic analysis were also reported by Kadirvel *et al.* (2013) who studied the diversity of thrips species in vegetable crops. Phylogenetic analysis was also done by Glover *et al.* (2010) where two distinct mtCOI clades were observed for *T. palmi.* Our findings are in line with Karimi *et al.* (2010) who observed that thrips sequences form six major clades including clades of *T. palmi* identified using DNA barcodes. *Thrips hawaiiensis* is common thrips species that damages the flower of various crop plants. In the present studies, this species is

reported from single location (Washim District) and found to be associated with cotton flower. This species was recently reported on cotton in Tamil Nadu (Amutha and Rachana, 2023). This species is reported as a strict flower-dwelling species (Reynaud *et al.,* 2008) and adults are polyphagous and it can feed on numerous host plants such as tobacco (Kurosawa *et al.,* 1964), rose (Woo and Paik, 1971), gladiolus (Chen and Lo, 1987), *Brassica oleracea* (Chandra and Lal, 1973), tea (Chen, 1979), mango (Lee and Wen, 1982) and banana (Tsai *et al.,* 1992; Jhala *et al.,* 2004).

Rhipiphorothrips cruentatus was identified from the Nagpur location. Till date, not a single report was available for this thrips species indicating cotton as one of the host plants in India. Instead, this species was recorded on plantation crops such as fruits and ornamental plants (Singh and Rachana, 2020). This is also new record that *R. cruentatus* dwells in cotton crop. *Ayyaria chaetophora* species was identified from three locations of Maharashtra. There are very scanty reports on this thrips species as well. Sanjta and Chauhan (2015) reported the association of this species with vegetable crops. In Thailand, Mabbett *et al.* (1984) reported that the majority of *A. chaetophora* are found on cotton. Common chilli thrips *S. dorsalis* was also reported from cotton crop in Chhindwara district of Madhya Pradesh. The presence of three rarely reported thrips species on cotton in India, namely *T. hawaiiensis, A. chaetophora* and *R. cruentatus,* offers a new dimension to the study.

The prior literature clearly reflected that *T. tabaci* was the dominant species infesting cotton in India but the present study represents abundance of *T. palmi* in cotton which might be correlated to the competitive displacement of former by later. However, the record of *T. palmi* on cotton is more significant in this context because there has been recent reports of this species on cotton in South India (Senguttuvan, 2019; Muppudathi *et al.,* 2020; Amutha and Rachna, 2023) and in North India (Rao *et al*., 2024). This study has also more significance by virtue of its propensity of spreading *tospoviruses* and *Illarviruses*. The establishment of *T. palmi* and its active role in spread of Tobacco Streak Virus (TSV) in cotton in Tamil Nadu was confirmed by Muppudathi *et al.* (2020). TSV is an emerging threat to cotton cultivation in India (Vinodkumar *et al.,* 2017) reported to cause up to 62.7% yield loss (Rageshwari *et al.,* 2017). TSV virus infestation in cotton may become a challenging task as no specific control strategies are available to contain virus diseases. Currently, TSV sporadically occurs in cotton crop in all the three major cotton growing zones of India but is gradually emerging as an important disease of cotton. In recent years, unprecedented increase in thrips population leading to adverse effect on crop productivity has been reported in several crops including cotton. In future, *T. palmi* may establish as dominant species of cotton and could possibly be posing threat to cotton cultivation in India. This might happen with phenomenon like ecological succession but it is not always a linear process, and disturbances can reset or alter the progression. Thrips compete for resources, including food and space on the cotton plants, leading to changes in their population density and distribution. The interactions between thrips and other organisms in the cotton ecosystem, such as predators, parasites, and pathogens, also play a role in shaping thrips populations. Factors such as environmental conditions, availability of food resources, natural enemies, and the resilience of the cotton plants all contribute to the dynamics of thrips populations. Disturbances may include environmental factors like extreme weather events, pesticide applications, or changes in agricultural practices.

Following a disturbance, the ecological succession of thrips in cotton may experience a reset, with potential changes in thrips populations and their interactions with the cotton plants. Understanding the ecological succession of thrips in cotton is crucial for developing effective pest management strategies that consider the complex dynamics of insect populations within agroecosystems. Integrated pest management (IPM) approaches that incorporate biological control, cultural practices, and judicious use of pesticides can help maintain a sustainable balance between thrips and cotton crops. Under the prevailing circumstances, the result of our study strongly supports the large share of *T. palmi* on cotton and thus demand more studies to track the dynamics of thrips population on cotton which may help to limit the upcoming potential havoc in cotton cultivation in India. An integrated pest management suggested by Nagrare *et al.* (2019) can be useful in minimising thrips population in cotton.

The present study revealed diversity of five thrips species viz. *Thrips palmi, Thrips hawaiiensis, Scirtothrips dorsalis, Ayyaria chaetophora* and *Rhipiphorothrips cruentatus* infesting cotton among which *T. palmi* was the predominant species, and demonstrating widespread habitation across the majority of cotton-growing districts. This comprehensive information serves as a valuable resource for monitoring the abundance and distribution patterns of thrips populations in the future. This knowledge is particularly significant in light of the prevalent transmission of Thrips-transmitted Tobacco streak virus (TSV) in cotton.

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