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Identification and Molecular Characterization of *Thrips palmi* (Karny 1925): A Potential GBNV Vector in Blackgram using ITS2 Marker

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ABSTRACT

Background: Identification and characterization of *T. palmi* a potential vector of GBNV in blackgram.

Methods: Pictorial taxonomic key-based morphological identification of *T. palmi* and molecular characterization through DNA sequencing using species specific marker ITS2 for accurate confirmation of the species.

Result: The present paper reports the host record of *T. palmi* on blackgram and its identification, molecular characterization through DNA barcodes from Andhra Pradesh. This study contributed *07* (seven) *novel gene sequences* to NCBI database and also revealed the existence of low genetic polymorphism among the ITS2 sequences of *T. palmi*. Species specific ITS2 primer was found promising. Six more genera of thrips were also identified *viz*. *Megalurothrips usitatus* (Bagnall), *Scirtothrips dorsalis* (Hood), *M. typicus* (Bagnall), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubuliferan thrips. *T. palmi* found in all GBNV hotspots of blackgram across Andhra Pradesh.

Key words: DNA barcodes, Genetic polymorphism, ITS2, *T. palmi*.

INTRODUCTION

India is the largest producer and also consumer of blackgram. It is referred as the “king of the pulses” due to its delicious taste and numerous other nutritional qualities (Vadivel *et al.*, 2023). It is rich in nutritional quality with 24-27% protein, 1% fat, 57% carbohydrate, 3.8% fibre and 4.8% ash. It is grown in both summer and winter seasons (Mohanlal *et al*., 2023). Furthermore, it is fed to milch cows in particular as nutrient-rich fodder. Globally India is the largest producer of black gram, accounting for more than 70% of production followed by Myanmar and Pakistan (Bharathi *et al*., 2025). Thrips, major sucking insect pests in blackgram causing considerable damage by sucking cell sap and also as vectors of GBNV which causes bud necrosis. A management technique requires accurate pest identification as a basic initial step. A technical person’s diagnosis depends on their ability to quickly and accurately identify taxa. Without the presence of adults, it is typically impossible to identify larval Thysanoptera to species level. A constraint in thrips morphological identification is that larval stages cannot be identified with most available keys and exhibit fewer characters of diagnostic value than the adults (Glover *et al.*, 2010). Species identification using morphological features has some significant limitations as the species might have minimal or no phenotypic changes but have high genetic variability. Morphological identification overlooks cryptic features, which are common in many groups and the use of keys requires a high level of expertise as misdiagnosis is common (Hebert *et al*. 2003; Armstrong and Ball, 2005). The most reliable identification is obtained by the combination of various techniques. The time-consuming nature of the classical morphological procedures does not make them unappreciated; particularly as appropriate identification using morphological keys is typically a necessary initial step in the validation of the more recent methods. Morphological identification is much cheaper economically than molecular identification as the materials and equipment used in morphological identification require less expenditure (Hillis and Davis, 1987; Wiens, 2004). Morphological keys may still be essential for specimen identification down to the genus level. Alternatively, these keys may be optional. Majority of thrips are host-plant specific,

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hence molecular and morphological identification techniques need to be used in a complementary manner to clearly identify the species of the specimens. Therefore, a more general, simple, accurate and large-scale identification method would be helpful to facilitate identification of thrips species occurring in a cropping system where multiple species co-exist, and the population dynamics are influenced by numerous factors (Kadirvel *et al.*, 2013). Molecular techniques provide powerful tools for the study of insect population ecology and insect systematics. In addition, analysis of molecular markers that can discriminate closely related species and monitor specific populations in the field. The outward traits of a species can vary within the species or overlap with those of other species, making the morphological examination method of adult identification challenging. The ''DNA barcoding'' is a method based on DNA sequencing of a standard gene region (Hebert *et al*., 2003). It can be helpful in species diagnosis because sequence divergences are usually much lower among individuals of a species than between closely related species (Hebert *et al*., 2003). However, studies on thrips infesting blackgram are scanty especially on their identification using morphology and molecular strategies together are very few. Keeping this in view current study was designed.

MATERIALS AND METHODS

The present investigation entitled "Identification and molecular characterization of *T. palmi* (Karny, 1925) a potential GBNV vector in blackgram using ITS2 marker" has been conducted in the laboratory of the Department of Entomology, Agricultural College, Bapatla, Guntur district, A.P., India during 2019-2022. Thrips were randomly collected from predominant blackgram cultivating areas duly covering all climatic zones of A.P., India. Thrips were collected by simply beating the plants on black tray and carefully transferred to vials containing alcohol, glycerin acetic acid (AGA) mixture having 10 parts of 60% ethyl alcohol with one part of glycerin and one part of acetic acid. Additional specimens collected were immediately transferred to -20°C to carryout molecular studies. Permanent mounts in natural Canada balsam were prepared for microscopic examination using Maceration and dehydration protocol (Mound and Kibby, 1998). Specimens were identified by following the taxonomic keys given by Cluever and Smith (2017), labeled neatly and then percentage of species composition was worked out. Further species confirmation was done through molecular characterization using stored buffer samples. From all the 35 locations, two samples each for *T. palmi* were subjected to molecular characterization through PCR. Further a representative sample from each district was selected and utilized for characterization studies. Single thrips specimens collected from each location was morphologically identified based on the taxonomic keys and quickly transferred to 1.5 mL centrifuge tubes with proper labeling. The DNA was extracted from a single thrips specimen using the salting out protocol given by Sunnucks and Hales (1996) with slight modifications (Supplementary material). Concentration of the DNA was examined, diluted and stored at -20°C for further PCR analysis. *Thrips palmi* specific marker *ITS2* (Internal transcribed spacer 2-located in the 5.8S region flanking the ITS2 region of ribosomal DNA) FP:GTGAACTG CAGGACACAT RP:CACC TGAA CAG AG GTCGG was employed. The PCR was carried out *Initial denaturation* 94°C~10 minutes; *Denaturation* 94°C~60 seconds; Annealing 55°C~60 seconds; Extension 72°C~60 seconds (total 35 cycles), Final extension 72°C~15 minutes; Hold ~4°C. The migration pattern of the DNA fragments in the agarose gel was visualized in a UV light transmitted gel documentation system (SYNGENE Gene flash, U.K.). After this, PCR products were processed for purification using QIAGEN QIAquick PCR Purification Kit (cat. No. 28104). A total of 07 (seven) samples were sequenced bidirectionally using Sanger di-deoxy chain termination method at Barcode Biosciences Pvt. Limited, Bengaluru. To investigate the genetic relationship across the collected samples a phylogenetic tree was constructed using all identified haplotypes. The homologous sequences were selected based on the similarity percentage between present study and sequences available at NCBI. Complete DNA alignment was done using MEGA software (version 11.0). Neighbor Joining tree (NJ) method with 1000 bootstrap was employed. Twenty-three gene sequences (ITS2) aligned against the current study sequences. The final dataset of thirty (30) sequences was aligned, edited using Bioeditv 7.0 software. To test the reciprocal monophyletic criteria for species identification, the generated sequence data set was further studied for genetic divergence through haplotype analysis. Diversity was estimated in terms of segregating sites, nucleotide and haplotype diversity along with Tajima's D statistic, which tests for neutrality and recent population expansion or contraction, using DNASP6.

RESULTS AND DISCUSSION

Specimens were identified based on the photo based key given by Cluever and Smith (2017) as follows:

1′ Wings (brachypterous or macropterous) or wing buds present 2

2′ Wings fully formed, with setae present .... Adult, 4

4′ Abdominal segment X conical; female with saw-like ovipositor .....Terebrantia, 6

6′ If ctenidia are present on abdominal tergites V–VII, ctenidium on tergite VIII posterior to spiracle; anterior margin of prothorax lacking major setae antennae 7-, 8-, or 9-segmented 15

15′ Lateral margins of abdominal tergites VI–VI lacking closely spaced rows of microtrichia; cilia of forewing fringe wavy; ocellar III setae not arising within the ocellar triangle.....16

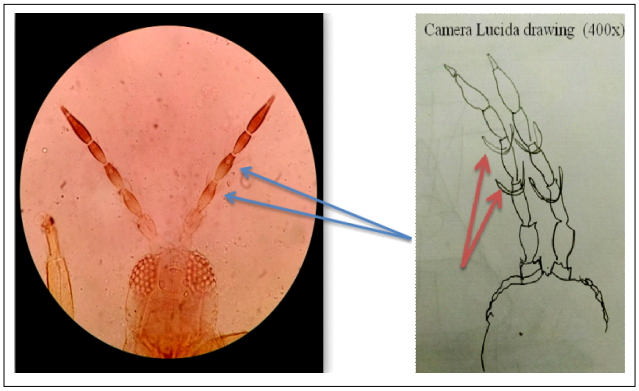


Plate 1: Antennal segments III and IV, forked sense cones.

16′ Abdominal tergites V–VIII with paired ctenidia laterally; antennae 7-or 8 segmented……19

19′ Tergites III-VIII lacking craspedum; pronotum transverse; antennae 7-or 8-segmented……20

20′ Metanotum with median pair of setae arising posterior to anterior margin; antennae 7 segmented……22

22′ Abdominal sternites lacking discal setae, setae present only at posterior margin; row of setae on 1st vein with spaces between setal bases much greater than length of each seta……23

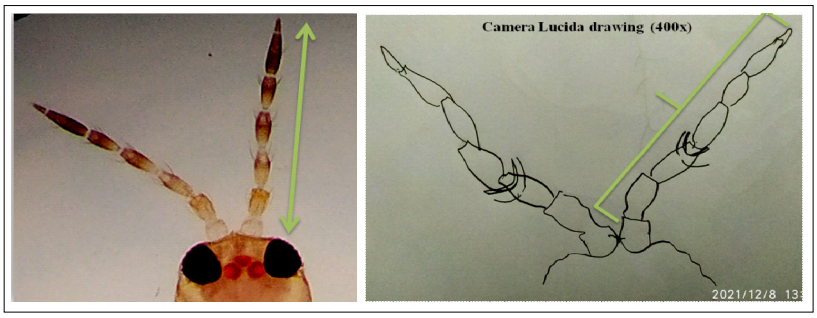


Plate 2: Antennae always seven-segmented.

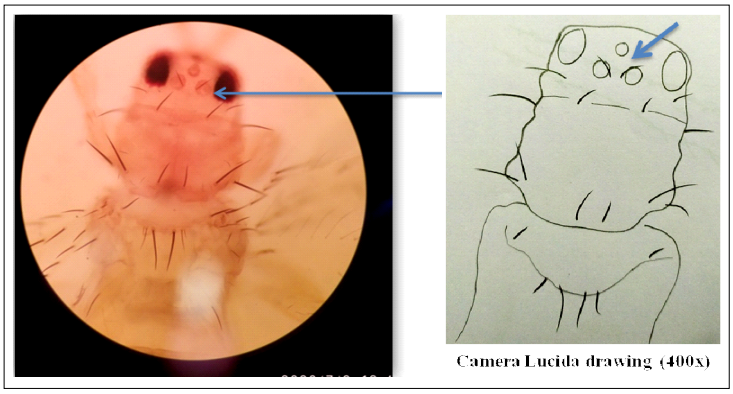


Plate 3: Ocellar setae III standing either just outside the ocellar triangle or touching the tangent lines connecting the anterior ocellus and each of the posterior ocelli.

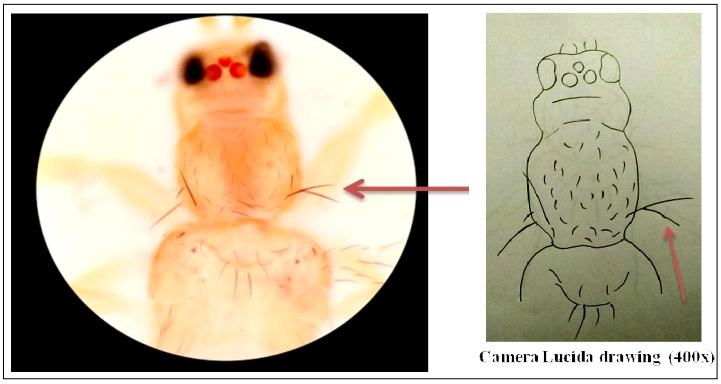


Plate 4: Pronotum, two pairs of major posteroangular setae.

23′ Metanotal campaniform sensilla present; microtrichia lacking on lateral thirds of tergites IV–VI .....*Thrips palmi* (Karny)

***Thrips palmi* (Karny)**

A clear yellow body with no dark areas on the head, thorax or abdomen (slightly thickened-blackish body setae); antennal segments I and II pale, III yellow with apex shaded

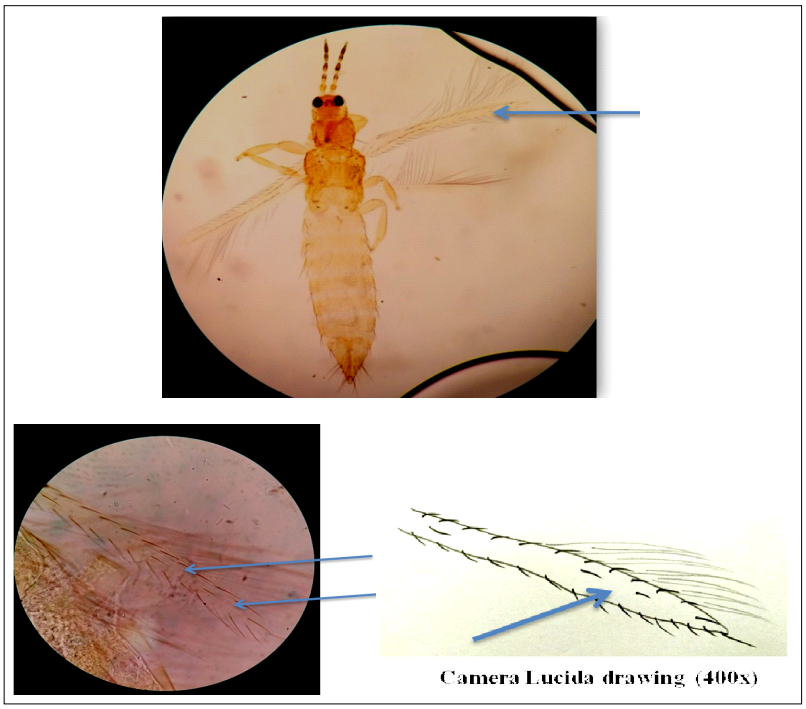


Plate 5: Forewing, first vein-three setae with gaps in the distal half.

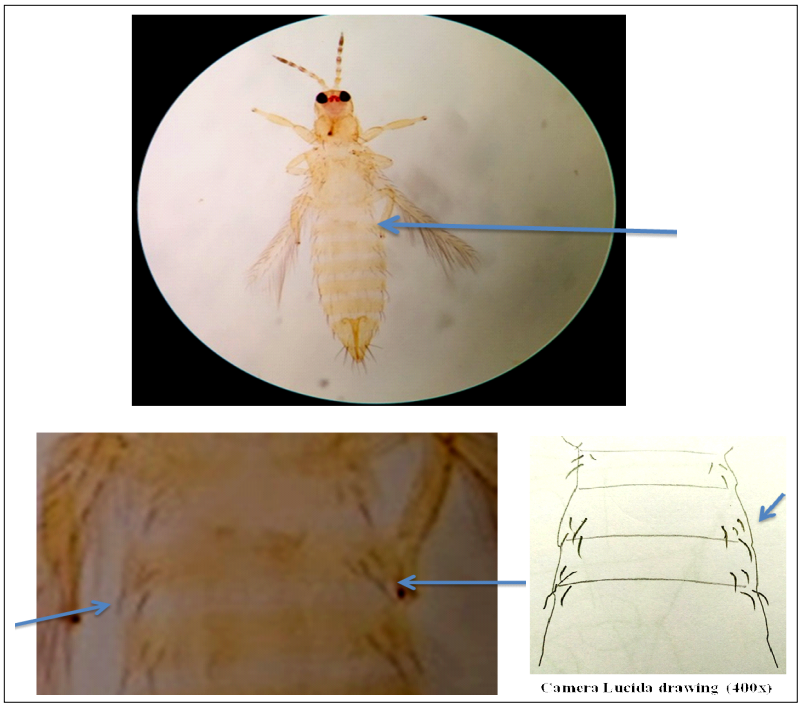


Plate 6: Abdominal tergite II, four lateral marginal setae.

Table 1: Distribution of *Thrips palmi* in blackgram in Andhra Pradesh, India based on morphological identification and corresponding GenBank Acc. numbers.

District	Geographic location and coordinates			*T. palmi* (per cent mean)	Molecular characterization	Sample code	GenBank Acc. number
Srikakulam	Etcherla	18.2412584	83.8447353	61.11	TP1*	LRTM 1	MZ427914
	Ponduru	18.3532918	83.7834948	78.95			
	Sigadam	18.3701058	83.6845722	60.00			
	R.Amudalavalasa	18.5608624	83.7368810	43.75			
	Rajam	18.4813227	83.7606665	81.25			
		Total per cent mean		65.01			
Vizianagaram	Gantyada	18.1430914	83.2878155	61.11			
	Bondapalli	18.2042411	83.3754091	66.67			
	Mentada	18.3388977	83.2981347	28.57	TP15*	LRTM 2	MZ427915
	Dattirajeru	18.3659343	83.3438147	70.59			
	Gajapathingaram	18.2848787	83.3891682	80.00			
		Total per cent mean		61.39			
Krishna	Avanigadda	16.0116513	80.9090502	35.00	TP21*	LRTM 3	MZ427916
	Challapalli	16.0888576	80.9127349	35.29			
	Mopidevi	16.1262959	80.9325017	60.00			
	Pamarru	16.3040357	80.9583395	43.75			
	Movva	16.2309489	80.9190293	72.22			
		Total per cent mean		49.25			
Guntur	Pittalavanipalem	15.9961473	80.6237128	33.33			
	Amarthalur	16.0816675	80.6185431	50.00			
	Ponnuru	16.0460922	80.5393197	52.94			
	Chebrolu	16.2453405	80.4994681	40.00			
	Tadikonda	16.3699950	80.4239710	43.75	TP39*	LRTM 4	MZ427917
		Total per cent mean		44.00			
Prakasam	Vetapalem	15.7739453	80.3451074	86.67			
	Chirala	15.870279	80.3013510	73.68			
	Ongole	15.5489679	80.0676630	68.42	TP45*	LRTM 5	MZ427918
	Chinaganjam	15.6960545	80.2304650	83.33			
	Naguluppalapadu	15.5793362	80.1429734	70.59			
		Total per cent mean		76.54			
Kurnool	Gospadu	15.3826723	78.4295505	88.2			
	Panyam	15.5027870	78.4008180	80.00			
	Bandiatmakuru	15.5915330	78.5129326	86.67			
	Banaganapalle	15.3010060	78.2669780	59.09	TP57*	LRTM 6	MZ427919
	Gadivemula	15.6746161	78.4230823	66.67			
		Total per cent mean		76.1			

Table 1: Continue...

Table 1: Continue...

Chittoor	Gudipala	13.1106230	79.1555510	93.33			
	Thavanampalle	13.2410380	79.0100890	73.91			
	Palasamudram	13.1660870	79.3601640	60.00	TP65*	LRTM 7	MZ427920
	Ganghadharnellore	13.2233630	79.2313280	54.55			
	Airala	13.4000562	79.0051720	53.85			
		Total per cent mean		67.13			
Andhra Pradesh		Total per cent mean		63.12			

Note: *Denotes the sample used for sequencing.

and sensorium forked (Plate 1), IV-VII brown but usually with base of IV-V yellow; forewings uniformly slightly shaded, prominent setae dark.

Antennae always seven-segmented (Plate 2).

Post ocular setae II and IV much smaller than remaining setae.

Ocellar setae III standing either just outside the ocellar triangle or touching the tangent lines connecting the anterior ocellus and each of the posterior ocelli (Plate 3).

Pronotum with transverse carina parallel to posterior margin, median area weakly transversely reticulate; 2 pairs of long posteroangular setae, outer longer than inner, one pair of anteroangular setae moderately prominent (Plate 4).

Metascutum with sculpture converging posteriorly; median pair of setae behind anterior margin; paired campaniform sensilla present.

Forewing first vein with three (occasionally two) distal setae (Plate 5).

Abdominal tergite II with four lateral marginal setae (Plate 6).

Abdominal tergites III to IV with setae S2 dark and subequal to S3.

Abdominal tergite VIII with posteromarginal comb in female complete, in male broadly developed posteriorly.

Abdominal tergite IX usually with two pairs of campaniform sensilla (pores).

Abdominal sternites without discal setae or ciliate microtrichia.

Abdominal pleurotergites without discal setae.

Male: Sternites III-VII each with a narrow transverse glandular area.

From Table 1, it was evident that *T. palmi* was found in all locations with 65.01, 61.39, 49.25, 44.00, 76.54, 76.13 and 67.13 per cent in Srikakulam, Vizianagaram, Krishna, Guntur, Prakasam, Kurnool and Chittoor districts of A.P., respectively. In Chittoor district *Megalurothrips typicus* (Bagnall), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubulifera thrips were also observed in meager numbers.

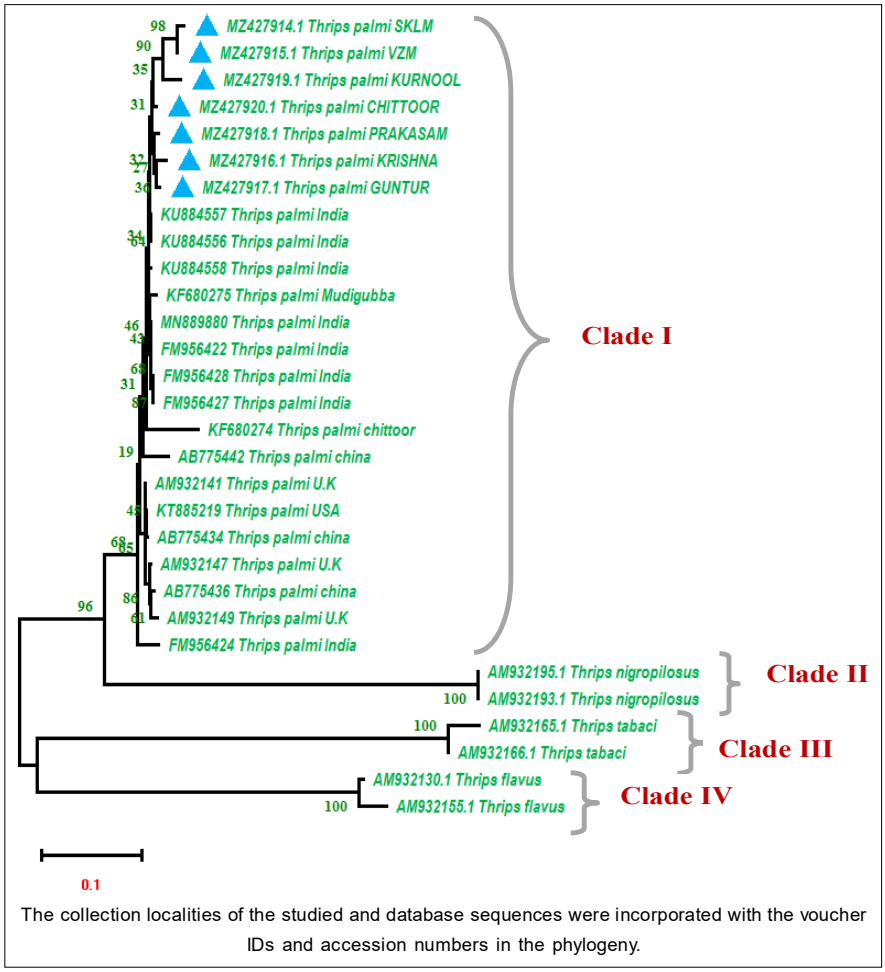
Confirmation of *Thrips palmi* using ITS2 marker

Internal Transcribed Spacer (ITS) is a challenging marker, technically it is present in multiple distinct copies and has likelihood of containing high intra and inter-genomic variation. This marker is useful for species identification in taxon specific studies as it produces alignment overlaps in the genus-specific range (Dentinger *et al.* 2011; Stern *et al.*, 2012). However, the fact that ITS2 sequences are potential markers for general phylogenetic studies and have been widely used for phylogenetic tree reconstructions both at genus and species levels which makes them ideal for species differentiation (Miao *et al.,* 2008; Schultz and Wolf, 2009). The ITS-based markers have been used by various researchers for species-level identification of thrips (Farris *et al*., 2010; Grazia *et al*., 2016; Toda and Komazaki, 2002; Kumar *et al*., 2017). All the 70

thrips samples collected from 35 geographic locations produced an amplicon size of ~570 bp with ITS2 marker. The results are in agreement with Sumit *et al*. (2020) who have reported that *T. palmi* was identified from the samples collected from brinjal, lettuce and tomato using multiplex PCR assay with designed ITS2 primer without any cross reactivity. Other workers Nakahara and Minoura (2015) amplified the internal transcribed spacer 2 region (ITS2) of nuclear ribosomal DNA using five specific primers for 71 individuals of the four thrips species *viz., T. palmi, T. tabaci, F. intonsa* and *F. occidentalis* that were frequently found in Japanese quarantine inspection sites based on species-specific single bands (470 bp, 410 bp, 370 bp, 280 bp). Yeh *et al*. (2014) obtained 43 ITS1 sequences ranging from 800-1200 bp for 15 thrips species and deposited in NCBI (AB904169-AB904212) and also reported that multiplex PCR using specific primers based on ITS1 sequences is a simple, reliable and cost-effective diagnostic tool in the identification of thrips (*T. tabaci*, *F. intonsa* and *S. dorsalis*). Farris *et al*. (2010) studied 432 *S. dorsalis* specimens representing 15 geographic populations and reported that 12 populations displayed 100% amplification of ITS2 fragment ranging from 131 to 135 bp and these included the populations from India, Japan, U.S.A, Barbados, Israel and Venezuela.

Sequencing and homology studies

Seven representative *T. palmi* samples (Table 1) were sequenced bidirectionally using sanger sequencing method. Homologous sequences across the globe were retrieved from NCBI database using BLASTN tool and subjected to homology studies. Our sequences had shown similarity of 97 to 100 per cent with data sets throughout the world. The NCBI data base sequences MN889880, KU884558, FM956428, FM956427, FM956422 from India, AB775442 from China showed 100% similarity with present *T. palmi* isolates. KF680274, KU884557, KU884556 sequences have shown 99 per cent similarity where as KF680275 (India), KT885219 and KT885218 (U.S.A), AB775439 and AB775435 (China) have shown 98 per cent similarity. MN1942020 (India), KT885216 (U.S.A), AM932178 and AM932146, AM932140, AM932157 (U.K), AB063341 (Japan), AB775437, AB775436 (China), KM877305 and LC416224 (Taiwan) have shown 97 per cent similarity. The sequences of the present study were aligned using MEGA



The collection localities of the studied and database sequences were incorporated with the voucher IDs and accession numbers in the phylogeny.

Fig 1: Neighbor joining phylogenic tree for *Thrips palmi* (bootstrap replicates 1000).

Table 2: Genetic diversity and Tajima's D evaluated for *Thrips palmi* specimens.

Species	No. of sequences	No. of segregating sites	No. of parsimony-informative sites	Nucleotide diversity (π)	Total no. of mutations	Standard deviation of π	Total no. of haplotypes	Haplotype diversity (Hd)	Standard deviation of haplotype diversity	Tajima's D (Calculated using the total number of mutations)
T. palmi	48	88	87	0.02836	96	0.00302	22	0.968	0.009	-1.07506 Not significant, P>0.10

11.0 (Molecular Evolutionary Genetic Analysis) with known reference sequences in NCBI website. Sequences generated in this study were annotated and submitted to the global database (GenBank) to acquire the unique accession numbers Table 1. Phylogeny tree was constructed using neighbor joining (NJ, ML) method depicted cohesive clustering of the identified seven sequences of *T. palmi* along with the database sequences (23 homologous sequences with similarity of 97 to 99%) with 1000 bootstrap replicates. The phylogenetic tree represented four distinct clades of the present dataset Clade-1, Clade II, Clade-III and Clade IV (Fig 1) with a bootstrap value >80% highlighting a significant rate of phylogenetic relationships among the species studied. Clade I showed cohesive clustering of present study specimens (accession number MZ427914 to MZ42720) with other reported isolates from Indian sub-continent. Further, *T. palmi* isolates from foreign countries were also clustered in clade I on different node. Isolates of *T. palmi* from China, U.K, U.S.A were very closely clustered in another node under clade I (*T. palmi* group), whereas the clade II comprised of *T. nigropilus* species of U.K, clade III comprised of *T. tabaci* species of U.K and clade IV comprised of *T. flavus* species of U.K. The out-group sequences were procured from NCBI website (https://www.ncbi.nlm.nih.gov). Out group is more distantly related group of organisms that serves as a reference group when determining the evolutionary relationships of the ingroup. Out-group serves as a point of comparison for the in-group and specifically allows for the phylogeny to be rooted. The phylogeny revealed the close relationship among India, china, U.K and U.S.A populations of *T. palmi*. Further, *T. palmi* species has close association with *T. nigropilosus* rather than with *T. tabaci* and *T. flavus* populations. Both the species were present in different clades under same cluster. Similar findings of distinct species-wise groups of *T. palmi*, *T. tabaci*, *F. occidentalis*, *S. dorsalis* and an unclassified group were also reported by (Kadirvel *et al*., 2013). Higher intra specific genetic variation was observed in case of *S. dorsalis* and *T. palmi* followed by *T. tabaci* and *F. occidentalis*. Genetic divergence and haplotype analysis study revealed that the seven ITS2 sequences of *T. palmi* of present study combined with 17 sequences from GenBank *i.e.,* four geographic regions (India, U.K, U.S.A and China) revealed 22 haplotypes which were clustered in a network according to genetic diversity existed among them. The ITS2 sequence with 552 nucleotide region was selected for the present haplotype analysis and finally 533 nucleotides were used excluding sites with gaps or missing data. Data pertaining to haplotype and genetic diversity is presented in Table 3. Data presented in the Table 2 and 3, (Fig 2) reveals that the present study sequences were formed into seven haplotypes namely Hap_1 to Hap_7 where as other ITS2 sequences from India were formed into seven haplotypes *i.e.* Hap_8 to Hap_14. Sequences from other countries *viz.* U.S.A and U.K were formed into Hap_15. The sequences from country

Table 3: Haplotype data (*Thrips palmi*).

Haplotype	GENBANK accession numbers	Source of sequence used	Geographic location of sequences
Hap_1	2 [MZ427914.1-1 MZ427914.1-2]	Present study	Srikakulam
Hap_2	2 [MZ427915.1-1 MZ427915.1-2]	Present study	Vizianagaram
Hap_3	2 [MZ427916.1-1 MZ427916.1-2]	Present study	Krishna
Hap_4	2 [MZ427917.1-1 MZ427917.1-2]	Present study	Guntur
Hap_5	2 [MZ427918.1-1 MZ427918.1-2]	Present study	Prakasam
Hap_6	2 [MZ427919.1-1 MZ427919.1-2]	Present study	Kurnool
Hap_7	2 [MZ427920.1-1 MZ427920.1-2]	Present study	Chittoor
Hap_8	2 [KF680274-1 KF680274-2]	NCBI	India, A.P
Hap_9	4 [MN889880-1 MN889880-2 FM956422-1 FM956422-2]	NCBI	India
Hap_10	2 [KU884558-1 KU884558-2]	NCBI	India
Hap_11	3 [FM956428-1 FM956428-2 FM956427-1]	NCBI	India
Hap_12	4 [KU884557-1 KU884557-2 KU884556-1 KU884556-2]	NCBI	India
Hap_13	1 [FM956427-2]	NCBI	India
Hap_14	2 [KF680275-1 KF680275-2]	NCBI	India
Hap_15	4 [KT885219-1 KT885219-2 AM932141-1 AM932141-2]	NCBI	U.S.A, U.S.A, U.K, U.K respectively
Hap_16	1 [AM932147-1]	NCBI	U.K
Hap_17	1 [AM932147-2]	NCBI	U.K.
Hap_18	2 [FM956424-1 FM956424-2]	NCBI	U.K.
Hap_19	2 [AB775434-1 AB775434-2]	NCBI	China
Hap_20	2 [AB775436-1 AB775436-2]	NCBI	China
Hap_21	2 [AB775442-1 AB775442-2]	NCBI	China
Hap_22	2 [AM932149-1 AM932149-2]	NCBI	U.K

Source: https://www.ncbi.nlm.nih.gov.

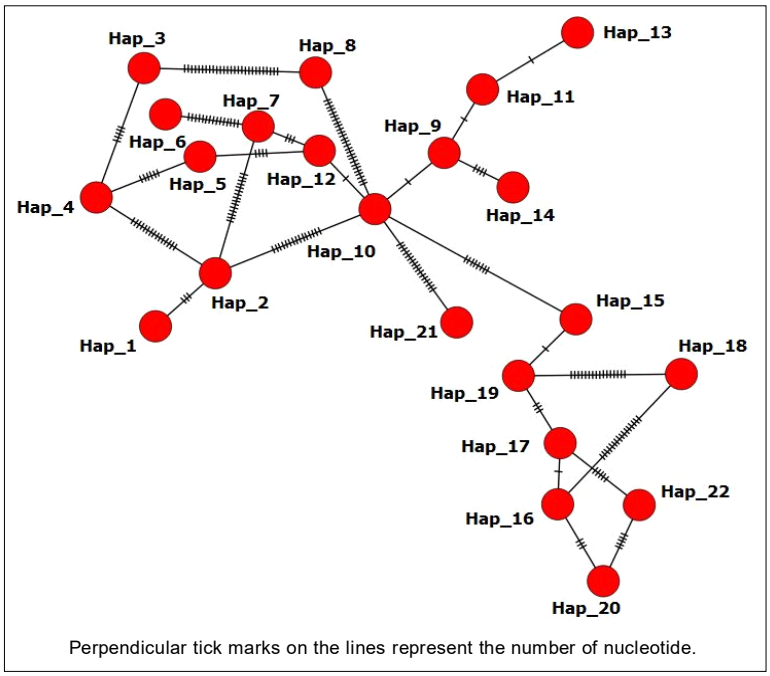


Fig 2: Haplotype network analysis of *Thrips palmi* using ITS2 sequences of present study and presumptive conspecifics from Genbank.

U.K were formed into three different haplotypes *i.e*., Hap_16, Hap_17, Hap_18 and Hap_22. Sequences from China were formed into three haplotypes *i.e.* Hap_19, Hap_20, Hap_21. The haplotype network generated in the present study is in accordance with the neighbor joining and maximum likelihood tree obtained earlier in this study. A total of 48 sequences were used for construction of haplotype network and these sequences were grouped into 22 haplotypes. A maximum of 88 segregating sites were observed with Nucleotide diversity (π) 0.02836 and standard deviation of nucleotide diversity (π) 0.00302. Haplotype diversity was recorded as 0.968 with a standard deviation of 0.009. Estimated mutations among the sequences were 96. Nucleotide diversity (pi) values range between 1 (very diverse) and 0 (not diverse) and hence present study showed pie value 0.00302 which revealed the existence of low genetic polymorphism among the ITS2 sequences of *T. palmi*. Tajimas D statistic was also estimated *i.e.,* -1.07506 (Not significant, P>0.10; values greater than +2 or less than -2 are likely to be significant). A negative Tajima's D signifies an excess of low frequency polymorphisms relative to expectation, indicating population size expansion where as a positive Tajima's D signifies low levels of low and high frequency polymorphisms, indicating a decrease in population size and/or balancing selection. However, such type of interpretation could not be made as D-value is statistically not significant.

CONCLUSION

The precise identification of species is a first step in development of management strategies for any pest. Species identification and subsequent understanding of vector specificity play a major role in management of vector transmitted diseases. Since thrips are very tiny insects, species identification by morphological observation is very difficult task and it may lead to confusion about the vector status. Hence, morphological identification coupled with molecular characterization gives accurate identification. As thrips are very minute, their total genomic DNA yield will be generally low. To overcome these possible problems of insufficient template, specific primers were used to amplify a smaller fragment of the targeted gene. The specific primer ITS2 and gene fragment-based DNA barcoding in the current study enabled proper identification of species *T. palmi*. The presence of remaining species were also in considerable range among the surveyed locations but not consistent.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Not applicable.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript. Informed consent. All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

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