A-6452
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RESEARCH ARTICLE Indian Journal of Agricultural Research

# Identification and Molecular Characterization of *S. dorsalis* (Hood) in Blackgram using mtCOIII Marker

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10.18805/IJARe.A-6452

## ABSTRACT

**Background:** Identification and characterization of *Scirtothrips dorsalis* in blackgram.

**Methods:** Pictorial taxonomic key-based morphology identification of *Scirtothrips dorsalis* and molecular characterization through DNA sequencing using species specific marker mtCOIII for accurate confirmation of the species.

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

**Key words:** DNA barcodes, Genetic polymorphism, mtCOIII, *S. dorsalis*.

## 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 causes bud necrosis. A management technique requires accurate pest identification as a basic initial step. Without the presence of adults, it is typically impossible to identify larval Thysanoptera to species. 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). Morphological identification is much cheaper economically than molecular identification as the materials and equipment used in require less expenditure (Hillis and Davis, 1987; Wiens, 2004). However, both molecular and morphological identification techniques need to be used in a complementary manner to clearly identify the species of the specimens. 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). Molecular techniques provide powerful tools for the study of insect population ecology and insect systematics. 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. ‘‘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. Our target species *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae: Thripinae),

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**How to cite this article:** Lella, R., Madhumati, T., Kumar, D.V.S., Kumari, V.P. and Roja, V. (2025). Identification and Molecular Characterization of *S. dorsalis* (Hood) in Blackgram using mtCOIII Marker. *Indian Journal of Agricultural Research*. 1-11. doi: 10.18805/IJARe.A-6452.

**Submitted:** 22-08-2025 **Accepted:** 09-10-2025 **Online:** 22-11-2025

commonly known as chilli thrips or yellow tea thrips, was described in 1919 from 34 females collected in India on castor and chillies by T.V. Ramakrishna during 13-14 March 1916 (Hood, 1919). Four synonymies are recognized, *Heliothrips minutissimus* (described from India), *Anaphothrips andreae* (Sumatra, Indonesia), *S. dorsalis* var. *padmae* (India) and *S. fragariae* (Australia) ( https://thrips.info/wiki/Scirtothrips_dorsalis ). Molecular studies investigating the phylogeny of *Scirtothrips* spp. in general (Hoddle *et al.,* 2008) and identification of *S. dorsalis* specifically (Rugman-Jones *et al.,* 2006), suggest that *S. dorsalis* is probably a mix of morphologically distinct and indistinguishable species that are only separable at the molecular level. Members of this species complex have different native ranges, and exhibit varying levels of polyphagy and invasion propensity (Dickey *et al.*, 2015a). Keeping this in view current study was designed.

## MATERIALS AND METHODS

The present investigation entitled "Identification and molecular characterization of *S. dorsalis* in blackgram using mtCOIII marker" has been conducted in the laboratory of 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 *via*ls 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) as follows:

1. Specimens were transferred to cavity block (40 mm × 40 mm) and added few drops of water to remove the traces of alcohol.
2. Slight abdominal cut was given to thrips (at intersegment membrane of 2nd and 3rd abdominal segments) under microscope to remove body contents.
3. Then the specimens were transferred to 3% NAOH solution and kept it for two hours.
4. Then, specimens were washed quickly using dropper for 2-5 min.
5. Specimens were passed through different grades of alcohol *i.e.* 50, 70, 90,100 per cent (5 min each).
6. Then the specimens were transferred to two mL centrifuge tube, contained bilayer of 100% ethanol and terpineol.
7. Specimens sinked in terpineol were used for study by placing on clean glass slide along with terpineol.
8. Small quantity of canada balsam was placed over the specimen.
9. Specimens were arranged in a desired manner to observe under microscope.
10. Slide mounts were kept in an oven at 35-40°C for three days.
11. Specimens were identified by following the taxonomic keys given by Hoddle and Mound (2003), labeled neatly mentioning details *viz.* host plant, date of collection, place of collection, sex, genus, species, collector's name.

Later 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 *S. dorsalis* 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. DNA was extracted from a single thrips specimen using the salting out protocol given by Sunnucks and Hales (1996) with slight modifications as follows. Alcohol dipped samples were dried for two minutes and transferred on to micro centrifuge tubes.

1. 100 µL TNES buffer was added to the sample.
2. Thrips were crushed carefully using sterile tooth pick
3. 1.7 µL of protienase k (10 mg/mL) was added after crushing then kept on vortex for five minutes to shake vigorously.
4. Samples were kept for overnight incubation (18 hrs). Vertexing of the sample was done for every two to three hours interval.
5. 28 µL of 5 M NaCl was added and then shaken vigorously. After mixing, kept for centrifugation at 13000 rpm for five minutes.
6. Supernatant was collected and transferred to fresh micro centrifuge tube, then the pellet was discarded.
7. Equal volume of 100 per cent ice cold ethanol was added and slowly mixed until the white flakes appeared.
8. Sample was kept at -20°C for one hour and then brought to the room temperature.
9. Supernatant was decanted and 70 per cent chilled ethanol (400 µL) was added to the pellet for washing.
10. Centrifugation was done (-4°C) at 13000 rpm for five minutes.
11. Supernatant was decanted carefully and 400 µL of absolute alcohol was added.
12. Supernatant was decanted carefully; the pellet was collected and dried at room temperature.
13. The pellet was dissolved in nuclease free water.

Upon completion, concentration of the DNA was examined, diluted and stored at -20°C for further PCR analysis. *S. dorsalis* specific marker *mt* COIII (mitochondria cytochrome oxidase III) FP: GTTCCATTTCATTTAG-TTTCACC; RP: GTCATACTACGTCAACAAAATGTC was employed (713 bp). PCR was carried out *Initial denaturation* 94oC ~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.). PCR products were purified 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, Bangalore. 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 has been employed. The NCBI deposited sequences of the present study were around 660 to 700 bp of mtCOIII. But the availability of similar sequences for this gene was of around 200 bp. Hence, a careful alignment of twenty final dataset mtCOIII gene sequences was done using Bioedit 7.2 version (Clustal-W) and finally arrived with 212 bp length. Further these sequences were assembled and aligned using the muscle DNA option in MEGA (11.0 version). Meager number of mtCOIII gene sequences of *S. dorsalis* were available in NCBI data base, hence whole genome sequences were also utilized in this tree construction. To test the reciprocal monophyletic criteria for species identification, the generated sequence data set was further studied for genetic divergence through haplotype analysis. 97-99 per cent similar sequences from NCBI website were used for haplotype network analysis. These sequences were aligned in MEGA 11.0 version using clustalW alignment option. GenBank sequences with clearly incorrect species assignments or potential contaminants (returning unexpected alignments or distances) were removed from the analysis. Sequence alignment (fasta file), for each species, was imported into DnaSP6 to reconstruct haplotypes and generated a haplotype data file (nexus). The nexus file was edited to add haplotype country links and a minimum spanning network (MSN) (Bandelt *et al.,* 1999) was constructed in PopART (http://popart.otago.ac.nz) to examine the relationships among haplotypes for each thrips species from different locations. The minimum spanning network was based on a minimum spanning tree where a set of sequence types connects all given types without creating any cycles or inferring additional (ancestral) nodes, such that the total length (*i.e.*, the sum of distances between linked sequence types) is minimal, allowing construction of the union of all minimum spanning trees (Bandelt *et al.,* 1999). Finally, the genetic 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

***Scirtothrips dorsalis* (Hood)**

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

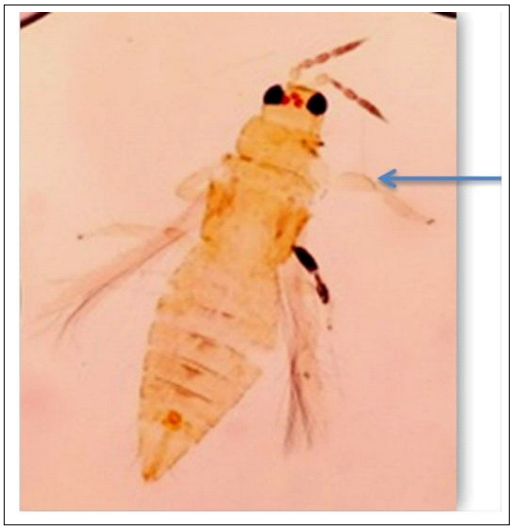
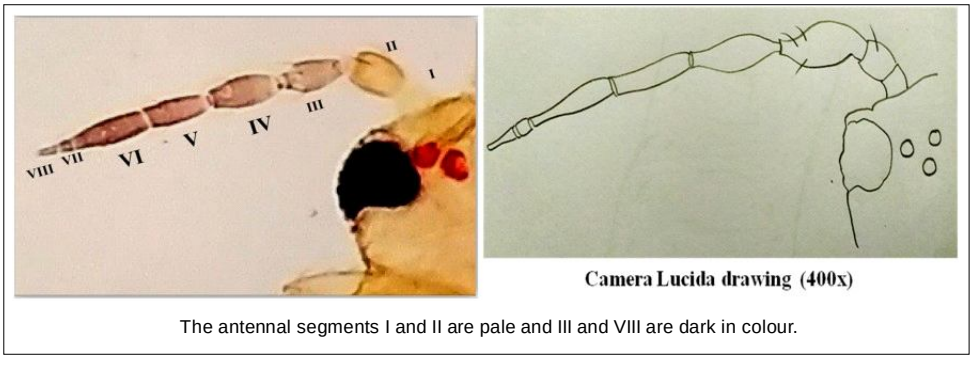


**Plate 1:** Pale colour head and legs of adult *S. dorsalis.*




The antennal segments I and II are pale and III and VIII are dark in colour.

**Plate 2:** The antennae are eight segmented.

15 (6′) Lateral margins of abdominal tergites IV-VI with microtrichia, rows of microtrichia closely spaced; cilia of forewing fringe straight; ocellar III setae arising between posterior ocelli, contained within ocellar triangle..... .....*Scirtothrips dorsalis* (Hood)

**Characteristic features of *S. dorsalis* (Hoddle and Mound, 2003)**

1. The head and legs of adult *S. dorsalis* are pale in colour (Plate 1).
2. Three pairs of ocellar setae are present on the head. The third pair is situated between posterior ocelli. The median postocular setae are two pairs and equal in length.
3. The antennae are eight segmented. The antennal segments I and II are pale and III and VIII are dark in colour (Plate 2).
4. The pronotum bears four pairs of posteromarginal setae. There is elongated reticulation on the middle of metanotum (Plate 3).

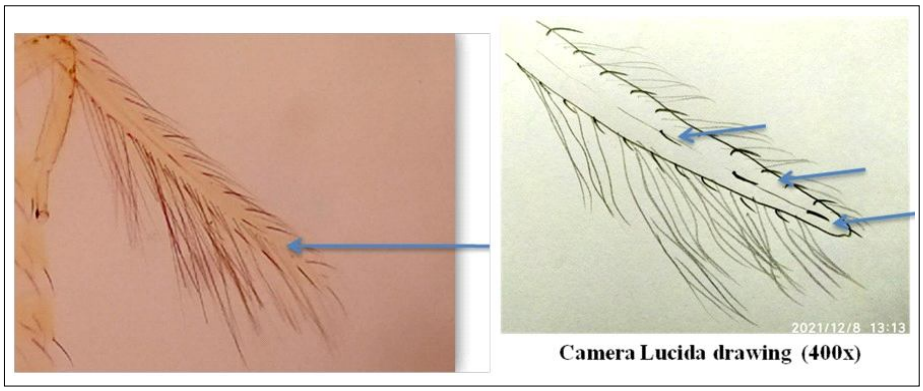



**Plate 3:** The first vein of forewing bears three irregular setae distally.

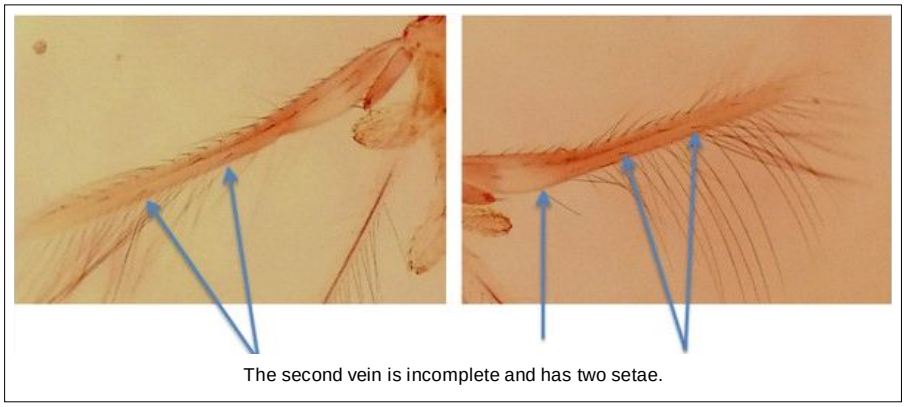



**Plate 4:** The forewing is distally light in color with straight cilia.

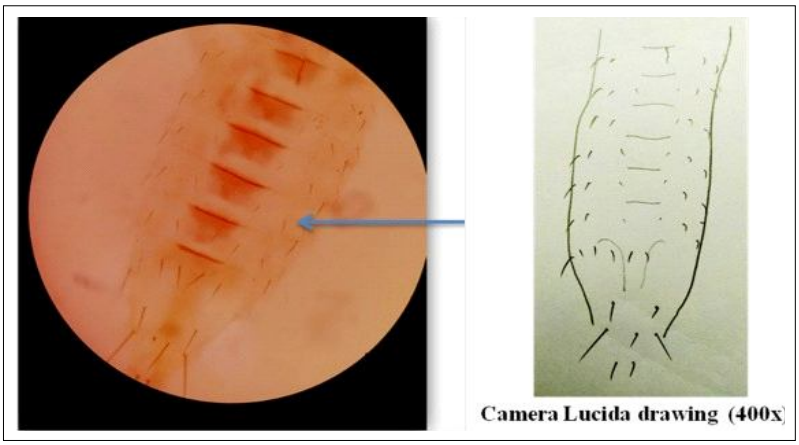



**Plate 5:** There are numerous microtrichia on the abdominal tergites.

5. The forewing is distally light in color with straight cilia. The first vein of forewing bears three irregular setae distally. The second vein is incomplete and has two setae (Plate 4).
6. There are numerous microtrichia on the abdominal tergites as well as sternites (Plate 5).
7. The tergites have a median dark patch. The antecostal ridges are also dark (Plate 6).
8. The tergal microtrichial fields of the abdomen have three discal setae. The posteromarginal comb on abdominal segment VIII is complete. In case of males, no drepanae is present on tergite IX.

Thrips specimens were also further confirmed by Dr. Laurence mound, honorary research fellow, CSIRO, Australia.

**Distribution of *S. dorsalis* on blackgram in A.P., India**

From the Table 1 it was evident that *S. dorsalis* was recorded with least mean per cent (6.83) in A.P., India. Out of thirty-five locations surveyed, no record of *S. dorsalis* was observed in 19 mandals *viz.*, Ponduru, R. Amudalavalasa and Rajam mandals of Srikakulam district, Gantyada, Bondapalli, Dattirajeru and Gajapatinagaram of Vizianagaram district, Pittalavanipalem and Chebrolu mandals of Guntur district, Vetapalem, Chinganjam, Naguluppalapadu of Prakasm district, Gospadu, Panyam, Bandi atmakuru and Gadivemula of Kurnool district, Gudipala, Thavanampalle, Airala of Chittoor district. Highest mean per cent of *S. dorsalis i.e.,* 52.38 was recorded in Mentada mandal of Vizianagaram district. In Chittoor district *Megalurothrips typicus* (Bagnall) (Plate 7), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubulifera thrips were also observed in meager numbers (Plate 8).

**Confirmation of *S. dorsalis* using mtCOIII marker**

Mitochondrial Cytochrome Oxidase III and ITS regions provided additional advantages at species-level identification due to larger interspecific distance than COI (Glover *et al.* 2010; Yeh *et al.* 2014). Low number of *S. dorsalis* species was identified in surveyed locations of Andhra Pradesh on blackgram during *rabi* 2019-20. In the present study a total of 17 individual specimens from different geographic locations were subjected to molecular characterization. *S. dorsalis* specimens were amplified with mtCOIII primer set and exhibited a clear amplification size of 713 bp. These results are in accordance with Sumit *et al.* (2020), who reported that the duplex PCR assay using combination of markers ITS2 and mtCOIII specific to *T. palmi* and *S. dorsalis*, respectively amplified DNA fragments of 568 bp and 713 bp size and discriminated between *T. palmi* and *S. dorsalis*. It was also reported that triplex PCR using a cocktail of primer pairs ITS2, mtCOIII and ITS2 amplified 568 bp, 713 bp and 388 bp products of *T. palmi*, *S. dorsalis* and *T. tabaci*, respectively. The multiplex PCR

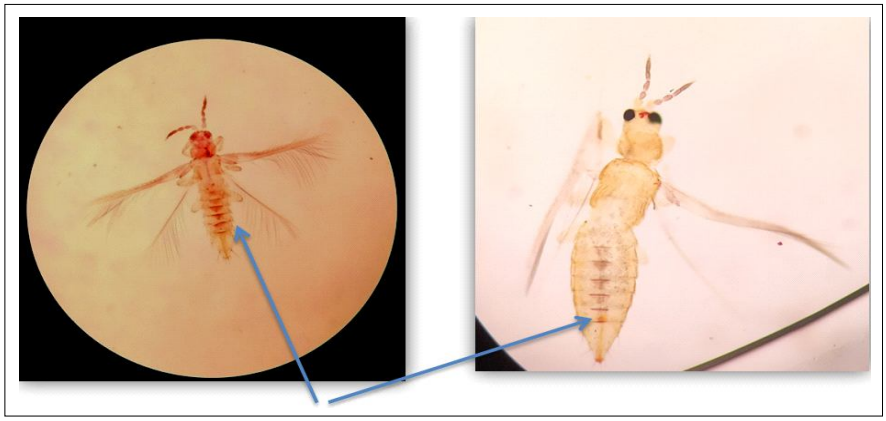


**Plate 6:** The tergites have a median dark patch.

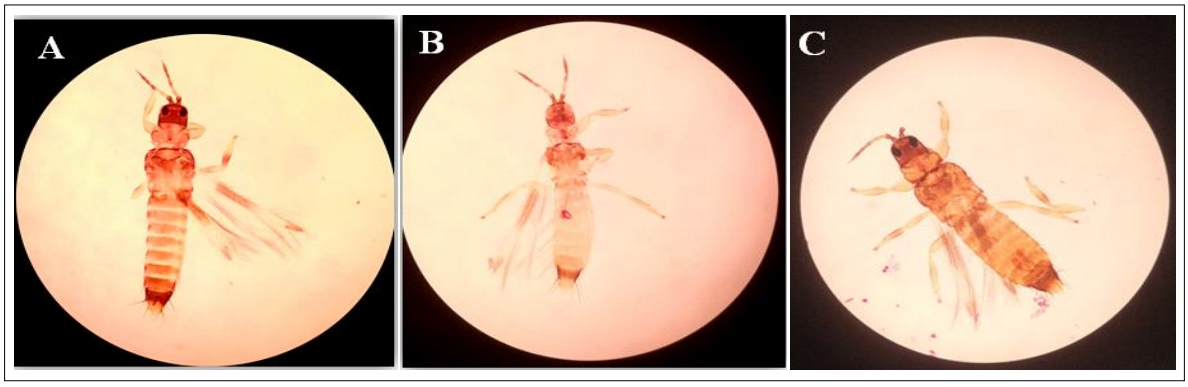



**Plate 7:** *Megalurothrips typicus.*

**Table 1:** Distribution of *S. dorsalis* in blackgram in A.P., India based on morphological identification and corresponding GenBank acc. numbers.

| District | Geographic location and coordinates | | | *S. dorsalis* (per cent mean) | Molecular characterization | Sample code | GenBank acc. number |
|---|---|---|---|---|---|---|---|
| Srikakulam | Etcherla | 18.2412584 | 83.8447353 | 11.11 | SD1* | LRTM 15 | MZ488494 |
| | Ponduru | 18.3532918 | 83.7834948 | 0.00 | | | |
| | Sigadam | 18.3701058 | 83.6845722 | 6.67 | SD2 | | |
| | R.Amudalavalasa | 18.5608624 | 83.7368810 | 0.00 | | | |
| | Rajam | 18.4813227 | 83.7606665 | 0.00 | | | |
| | | Total per cent mean | | 3.56 | | | |
| Vizianagaram | Gantyada | 18.1430914 | 83.2878155 | 0.00 | | | |
| | Bondapalli | 18.2042411 | 83.3754091 | 0.00 | | | |
| | Mentada | 18.3388977 | 83.2981347 | 52.38 | SD3*, SD4 | LRTM 16 | MZ488495 |
| | Dattirajeru | 18.3659343 | 83.3438147 | 0.00 | | | |
| | Gajapathingaram | 18.2848787 | 83.3891682 | 0.00 | | | |
| | | Total per cent mean | | 10.48 | | | |
| Krishna | Avanigadda | 16.0116513 | 80.9090502 | 15.00 | SD5* | LRTM 17 | MZ488496 |
| | Challapalli | 16.0888576 | 80.9127349 | 17.65 | SD6 | | |
| | Mopidevi | 16.1262959 | 80.9325017 | 13.33 | SD7 | | |
| | Pamarru | 16.3040357 | 80.9583395 | 18.75 | SD8 | | |
| | Movva | 16.2309489 | 80.9190293 | 11.11 | SD9 | | |
| | | Total per cent mean | | 15.17 | | | |
| Guntur | Pittalavanipalem | 15.9961473 | 80.6237128 | 0.00 | | | |
| | Amarthalur | 16.0816675 | 80.6185431 | 18.75 | SD10 | | |
| | Ponnuru | 16.0460922 | 80.5393197 | 11.76 | SD11 | | |
| | Chebrolu | 16.2453405 | 80.4994681 | 6.67 | | | |
| | Tadikonda | 16.3699950 | 80.4239710 | 6.25 | SD12* | LRTM 18 | MZ488497 |
| | | Total per cent mean | | 8.69 | | | |
| Prakasam | Vetapalem | 15.7739453 | 80.3451074 | 0.00 | | | |
| | Chirala | 15.870279 | 80.3013510 | 5.26 | SD13 | | |
| | Ongole | 15.5489679 | 80.0676630 | 10.53 | SD14* | LRTM 19 | MZ488498 |
| | Chinaganjam | 15.6960545 | 80.2304650 | 0.00 | | | |
| | Naguluppalapadu | 15.5793362 | 80.1429734 | 0.00 | | | |
| | | Total per cent mean | | 3.16 | | | |
| Kurnool | Gospadu | 15.3826723 | 78.4295505 | 0.00 | | | |
| | Panyam | 15.5027870 | 78.4008180 | 0.00 | | | |
| | Bandiatmakuru | 15.5915330 | 78.5129326 | 0.00 | | | |
| | Banaganapalle | 15.3010060 | 78.2669780 | 13.64 | SD15* | LRTM 20 | MZ488499 |
| | Gadivemula | 15.6746161 | 78.4230823 | 0.00 | | | |
| | | Total per cent mean | | 2.73 | | | |

**Table 1: Continue...**

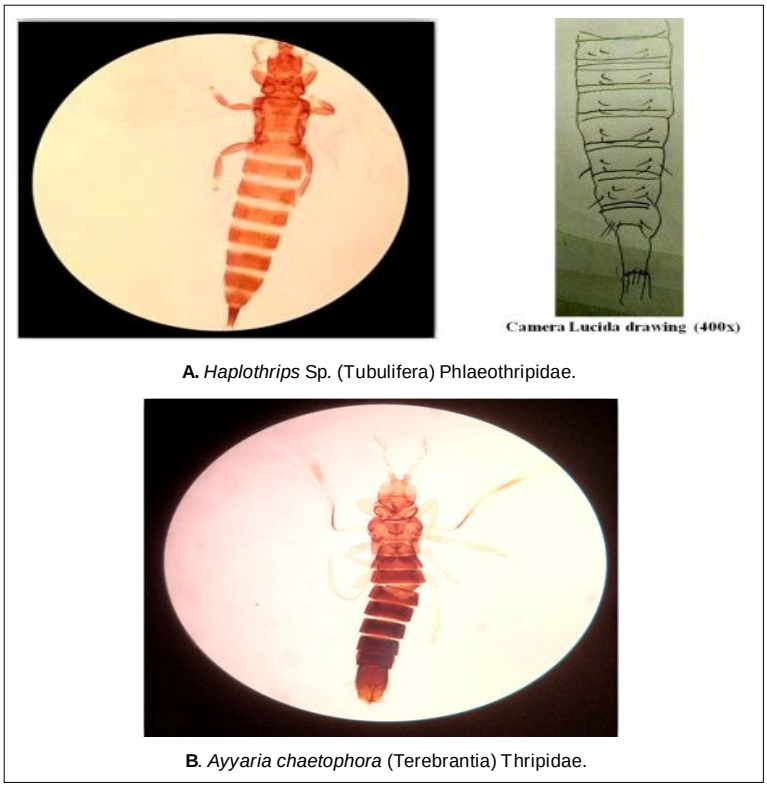
**Table 1: Continue...**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Chittoor | Gudipala | 13.1106230 | 79.1555510 | 0.00 | | | |
| | Thavanampalle | 13.2410380 | 79.0100890 | 0.00 | | | |
| | Palasamudram | 13.1660870 | 79.3601640 | 6.67 | SD16* | LRTM 21 | MZ488500 |
| | Ganghadharnellore | 13.2233630 | 79.2313280 | 13.64 | SD17 | | |
| | Airala | 13.4000562 | 79.0051720 | 0.00 | | | |
| | | Total per cent mean | | 4.06 | | | |
| Andhra Pradesh | | Total per cent mean | | 6.83 | | | |

Note: * Denotes the sample used for sequencing.

**Table 2:** Genetic diversity and Tajima's D evaluated for *S. dorsalis* specimens.

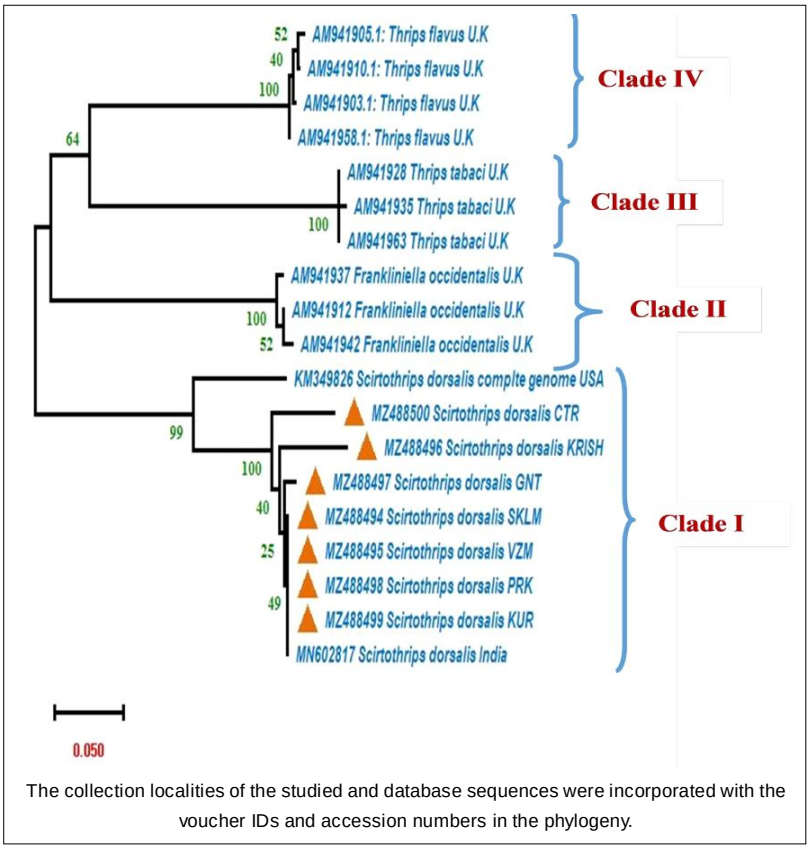
| Species | No. of sequences | No. of segregating sites | No. of parsimony-informative sites | Nucleotide diversity ($\pi$) | Total no. of mutations | Standard deviation of $\pi$ | Total no. of haplotypes | Haplotype diversity (Hd) | Standard deviation of haplotype diversity | Tajima's D (Calculated using the total number of mutations) |
|---|---|---|---|---|---|---|---|---|---|---|
| *S. dorsalis* | 20 | 43 | 43 | 0.04610 | 46 | 0.01303 | 6 | 0.7368 | 0.093 | -0.93034<br>Not significant, P>0.10 |




**A.** *Haplothrips* Sp. (Tubulifera) Phlaeothripidae.

**B**. *Ayyaria chaetophora* (Terebrantia) Thripidae.

**Plate 8:** Other thrips species identified.




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

**Fig 1a:** Neibhour Joining phylogenic tree for *S. dorsalis*, (bootstrap replicates 1000).

identified all the four thrips vectors *viz*. *T. palmi*, *S. dorsalis*, *T. tabaci* and *F. schultzei* using combination of ITS2, mtCOIII, ITS2 and ITS2 markers yielded products of 568 bp, 713 bp, 388 bp and 200 bp.

### Sequencing and homology studies

*S. dorsalis* sequences (mt COIII gene) of present study have shown 98 to 99 per cent similarity with the data base sequences belongs to India (Partial CDS-MN602817, MN193061; whole genome sequence KM349827). DNA sequences of *S. dorsalis* were annotated and submitted into the global database (GenBank) to acquire the unique accession numbers. List of samples, geographic location along with accession numbers obtained were presented in the Table 1.

### Phylogeny analysis

Neighbour Joining phylogenetic tree in Fig 1a and 1b segregated two species based on the reciprocal monophyly criteria and not to interpret phylogeny of genus *Scirtothrips*. The analysis of NJ tree yielded four major clades with high bootstrap support. The present study isolates (MZ488494 to MZ488500) along with other previously reported Indian isolate and whole genome sequences from U.S.A were very closely clustered under clade I formed as *S. dorsalis* group. The mtCOIII gene sequences generated from United Kingdom from NCBI database were clustered under clade II formed *Frankliniella occidentalis* group, clade III formed as *Thrips tabaci* group, clade IV formed as *Thrips flavus* group. Very few mtCOIII gene sequences of thrips species were available in the NCBI data base till today and the

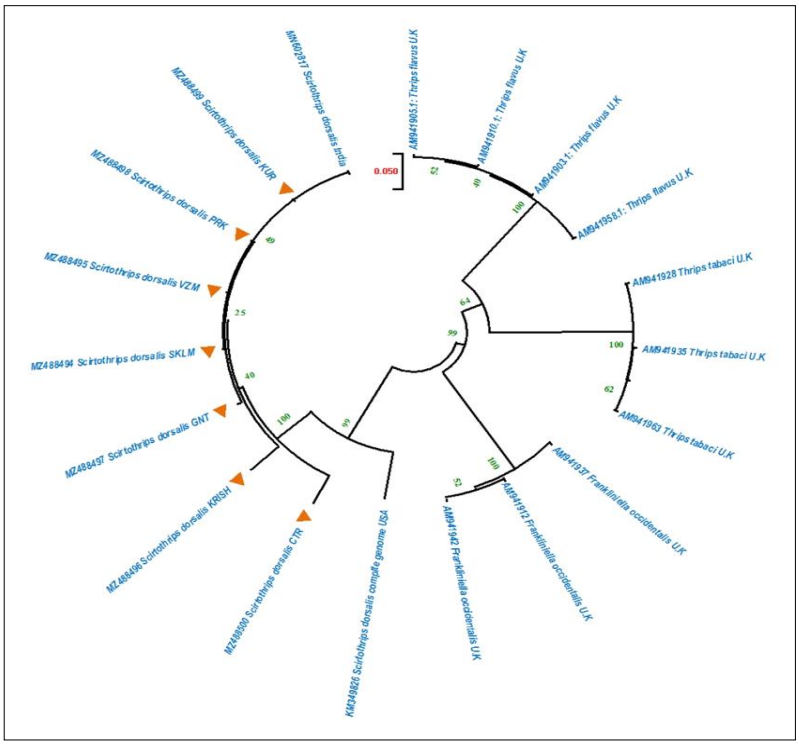



**Fig 1b:** Neibhour Joining phylogenic tree for *S. dorsalis*, (bootstrap replicates 1000).

**Table 3:** Haplotype data (*S. dorsalis*).

| Haplotype | Gen Bank accession numbers | Source of sequence used | Geographic location of sequences |
|---|---|---|---|
| Hap_1 | 10 [MZ488494-1 MZ488494-2 MZ488495-1 MZ488495-2 MZ488498-1 MZ488498-2 MZ488499-1 MZ488499-2 MN602817-1 MN602817-2] | Present study and NCBI | Srikakulam, Vizianagaram, Prakasam, Kurnool of present study and New Delhi from NCBI respectively. |
| Hap_2 | 2 [MZ488496-1 MZ488496-2] | Present study | Krishna |
| Hap_3 | 2 [MZ488497-1 MZ488497-2] | Present study | Guntur |
| Hap_4 | 2 [MZ488500-1 MZ488500-2] | Present study | Chitturu |
| Hap_5 | 2 [KM349826-1 KM349826-2] | NCBI | U.S.A |
| Hap_6 | 2 [KM349827-1 KM349827-2] | NCBI | U.S.A |

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

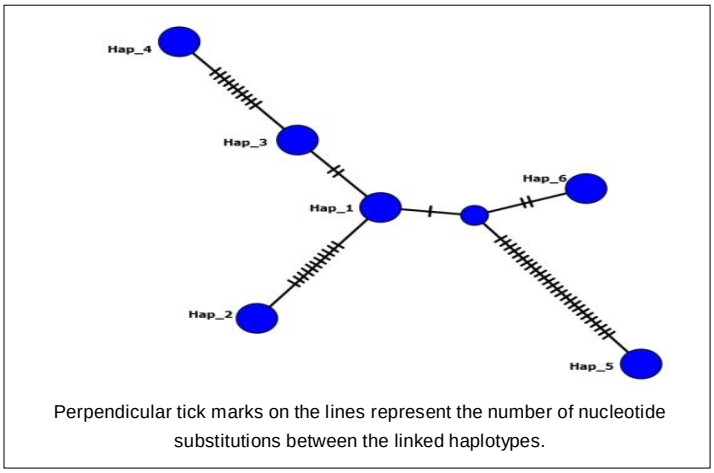



**Fig 2:** Haplotype network analysis of *S. dorsalis* using mtCOIII sequences of present study and presumptive conspecifics from Genbank.

present study generated mtCOIII sequence variants of *Scirtothrips dorsalis* from southern parts of India for the first time and deposited in NCBI. The findings of the present study are in accordance with Chakraborty *et al.* (2019) who studied both morphology and molecular approaches to delimit the selected *Scirtothrips* species from India, where 43 generated barcode sequences, six sequences of three species (*S. hitam, S. mangiferae* and *S. malayensis*) are the novel contribution in global database. The Bayesian (BA) phylogeny clearly distinguished all the studied species with reciprocal monophyletic criteria and represented multiple clades in *S. dorsalis* and *S. oligochaetus*. The high Kimura-2-Parameter (K2P) genetic divergences were observed between the multiple clades of *S. dorsalis* (4.5-8.8%) and *S. oligochaetus* (6.4%), which indicated possible existence of cryptic diversity. Development of morphological keys for six *Scirtothrips* species including *S. hitam* as a new record to India for the identification of those species.

**Genetic divergence and haplotype analysis**

Mitochondrial Cytochrome Oxidase III sequences of present study *i.e.,* seven sequences of *S. dorsalis* from Andhra Pradesh combined with three sequences procured from GenBank belongs to two geographic regions (India, U.S.A countries) revealed six haplotypes which were clustered in a network according to genetic diversity existed among them (Fig 2). The mtCOIII sequence with 216 nucleotide region was selected for the present haplotype analysis excluding sites with gaps or missing data. Data pertaining to haplotype and genetic diversity of *S. dorsalis* (Table 2 and 3) revealed that Srikakulam, Vizianagaram, Prakasam, Kurnool of present study and New Delhi from NCBI were formed into Hap_1 where as other mtCOIII sequences from Krishna, Guntur and Chittoor of present study were formed into Hap_2, Hap_3 and Hap_4, respectively. Two sequences from U.S.A were formed into Hap_5 and Hap_6, respectively. This haplotype network was in support with our previously constructed neighbor joining and maximum likelihood trees of the present study. A total of 20 sequences were used for haplotype network and these sequences were grouped into six haplotypes. A maximum of 43 segregating sites were observed with nucleotide diversity ($\pi$) 0.04610 and standard de*via*tion of nucleotide diversity ($\pi$) *i.e.* 0.01303. Haplotype diversity was recorded as 0.7368 with a standard de*via*tion of 0.093. Estimated mutations among the sequences were 46. Tajimas D statistic was 0.93034 (Not significant, P>0.10) which revealed the existence of low genetic polymorphism among the COIII sequences of *S. dorsalis*.

## 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 of thrips species. Hence, morphological identification coupled with molecular characterization gives accurate identification of a particular species.

## ACKNOWLEDGEMENT

The present study was conducted by the corresponding author during doctoral degree programme.

**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**

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.

### 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.

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