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# Identification and Characterization Through DNA Barcodes of bean Flower Thrips *Megalurothrips usitatus* (Bagnall) in Blackgram in South India

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## ABSTRACT

**Background:** Identification and characterization of thrips species in significant blackgram cultivation regions with bud necrosis disease as a key concern.

**Methods:** Morphological species identification through pictorial taxonomic keys and molecular characterization through DNA sequencing using PCR based techniques with species specific marker mtCOI for further confirmation of the species.

**Result:** The present paper reports the host record of *M. usitatus* on blackgram and its molecular characterization through DNA barcodes from South India for the first time. This study contributed a total of 07 novel gene sequences to NCBI. This study revealed the existence of low genetic polymorphism among the COI sequences of *M. usitatus*. Six more genera of thrips were also identified *viz*. *T. palmi*, *Scirtothrips dorsalis*, *Megalurothrips typicus* (Bagnall), *Ayyaria chaetophora* (Karny), *Phibalothrips peringueyi* (Faure) and some Tubuliferan thrips. Interestingly *Megalurothrips usitatus* is the second most predominant spp. infesting blackgram in all locations after *T. palmi*.

**Key words:** DNA barcodes, Genetic polymorphism, *Megalurothrips usitatus*, *mtCOI*.

## INTRODUCTION

Thrips are the major sucking insect pests in pulses, mainly on blackgram and greengram, causing considerable damage by sucking cell sap from different tender parts of plants and also acting as vectors of different plant viruses that cause leaf curl and bud necrosis, besides direct injury by feeding (Ananthakrishnan, 1980). 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. For instance, adult thrips are frequently confused with staphylinid beetles, while larval thrips are frequently misidentified as collembolan (Springtails) (Vierbergen 1995). Without the presence of adults, it is typically impossible to identify larval Thysanoptera to species. Though certain economically significant species are polyphagous, the majority of thrips are host-plant specific. Since these species have similar morphologies and are frequently spotted in fields together, it can be difficult to distinguish between them, particularly in the early stages of growth (Cheol *et al.,* 2006).

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. Furthermore, in order to validate the outcomes of novel identification strategies, morphological keys may still be essential for specimen identification down to the genus level. Alternatively, these keys may be optional. Molecular techniques provide powerful tools for the study of insect population ecology and insect systematics (Hoy, 2003).

In addition, analysis of mitochondrial DNA (mtDNA) is useful for providing molecular markers that can discriminate closely related species and monitor specific populations in a field (Simon 1991). The outward traits of a species can vary within the species or overlap with those

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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.* 2003b). 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.* 2003a).

## MATERIALS AND METHODS

### Sample collection and preservation and mounting of thrips specimens

Thrips have been collected from different significant blackgram growing regions in Andhra Pradesh, India (Annexure I). A completely impartial and strict randomization approach was adopted in a total of 35 locations. Thrips were carefully collected in *via*ls containing an alcohol-glycerin-acetic acid (AGA) mixture that contained 10 parts 60% ethyl alcohol, 1 part glycerin and 1 part acetic acid. Subsequently stored in 95% alcohol with cold packs at -20°C to conduct additional molecular research. The permanent mounts were prepared using Maceration and dehydration protocol (Mound and Kibby 1998). Slide mounts were stored in an oven at 35-40°C For 72 h. The specimens were labeled with all details including sex, genus and species. They were identified using the taxonomic keys provided by Mound and Yongfoo (2009), Hoddle and Mound (2003) and Cluever and Smith (2017). Percentage of species composition was calculated. Following the taxonomic identification up to the species level molecular analysis using buffer samples had been carried out to confirm the species.

**Annexure I:** Particulars of Villages and Mandals of Andhra Pradesh from which thrips specimens were collected on blackgram.

| District | Mandal | Village | Geographic coordinate | |
|---|---|---|---|---|
| Srikakulam | Etcherla | Nandigam | 18.2412584 | 83.8447353 |
| | Ponduru | Rapaka | 18.3532918 | 83.7834948 |
| | Sigadam | Chettupodili | 18.3701058 | 83.6845722 |
| | Regidi Amudalavalasa | Burada | 18.5608624 | 83.7368810 |
| | Rajam | Rajam | 18.4813227 | 83.7606665 |
| Vizianagaram | Gantayada | Gantayada | 18.1430914 | 83.2878155 |
| | Bondapalli | Nelivada | 18.2042411 | 83.3754091 |
| | Mentada | Meesalapeta | 18.3388977 | 83.2981347 |
| | Dattirajeru | Pedamanapuram | 18.3659343 | 83.3438147 |
| | Gajapathinagaram | Jinnam | 18.2848787 | 83.3891682 |
| Krishna | Avanigadda | Avanigadda | 16.0116513 | 80.9090502 |
| | Challapalli | Challapalli | 16.0888576 | 80.9127349 |
| | Mopidevi | Mopidevi | 16.1262959 | 80.9325017 |
| | Pamarru | Pamarru | 16.3040357 | 80.9583395 |
| | Movva | Movva | 16.2309489 | 80.9190293 |
| Guntur | Pittalavaniaplem | Chandole | 15.9961473 | 80.6237128 |
| | Amarthaluru | Pyaparru | 16.0816675 | 80.6185431 |
| | Ponnuru | Chinthalapudi | 16.0460922 | 80.5393197 |
| | Chebrolu | Narakoduru | 16.2453405 | 80.4994681 |
| | Guntur | Lam | 16.3699950 | 80.4239710 |
| Prakasam | Vetapalem | Akkayyapalem | 15.7739453 | 80.3451074 |
| | Chirala | Karamchedu | 15.870279 | 80.3013510 |
| | Ongole | Throvagunta | 15.5489679 | 80.0676630 |
| | Chinaganajam | Chinaganjam | 15.6960545 | 80.2304650 |
| | Naguluppalapadu | Ammanabrolu | 15.5793362 | 80.1429734 |
| Kurnool | Gospadu | M.chinthalkunta | 15.3826723 | 78.4295505 |
| | Panyam | Balapanur | 15.5027870 | 78.4008180 |
| | Bandiatmakuru | keerthireddipadu | 15.5915330 | 78.5129326 |
| | Banaganapalle | kaipa | 15.3010060 | 78.2669780 |
| | Gadivemula | Gadiyarevula | 15.6746161 | 78.4230823 |
| Chitturu | Gudipala | Krishnajimmapuram | 13.1106230 | 79.1555510 |
| | Thavanampalle | T.Puttur | 13.2410380 | 79.0100890 |
| | Palasamudram | Thirumalarajapuram | 13.1660870 | 79.3601640 |
| | Ganghadharnellore | Vepanjeri | 13.2233630 | 79.2313280 |
| | Airala | kaminayunipalle | 13.4000562 | 79.0051720 |

**Molecular characterization of *M. usitatus***

Two *M. usitatus* samples from each of the 35 locations underwent PCR molecular analysis. Additionally, a representative sample was chosen from each district and used in the characterization research. Single thrips specimens identified morphologically using taxonomic keys were subjected to salting out technique described by Sunnucks and Hales (1996). Following DNA concentration testing, based on the intensity of the sample, DNA samples were diluted and stored at -20°C for subsequent PCR analysis. PCR amplification was carried out with the set of primers *FP: GGTCAACAAATCATAAAGATATTGG RP: TAAACTTCAGGGTGACCAAAAAATCA*. In the present study *Megalurothrips usitatus*, *mt* COI (mitochondrial cytochrome oxidase I) markers were employed to amplify 5′ -end portion under set of PCR conditions (Annealing temperature 55°C, Amplicon size 655 bp). DNA was detected using agarose gel electrophoresis, following the procedure outlined by Sambrook *et al.* (1989). Amplified PCR products were separated on one per cent agarose gel in 1X TBE buffer at 100V. An ultraviolet light-transmitted gel documentation system (SYNGENE Gene flash, U.K.) was used to visualize the migrating pattern of the DNA fragments in the gel. Sequencing of amplified products, Phylogeny tree construction, Assessment of Haplotypes were done using specific protocols (Supplementary material).

## RESULTS AND DISCUSSION

**Key to *Megalurothrips usitatus* (Bagnall) given by (Mound and Yongfoo 2009)**

Never dark brown and reticulate; major setae not long and capitate. Pronotum never with more than five pairs of major setae. Abdominal tergites without numerous microtrichia occupying lateral thirds, with a few microtrichia near lateral margins. Major setae on head, pronotum and forewings setaceous Antennal segment II external margin not prolonged, segment I not swollen. Pronotum with at least one pair of prominent posteroangular setae Pronotal anterior margin with 1 or 2 pairs of setae that are much longer than discal setae. Forewing first vein with setal row widely interrupted; tergites without ctenidia forewing second vein with many equally spaced setae; tergite VIII either with no comb or with comb interrupted medially; tergites and sternites without prominent reticulation. Ocellar setae pair III arising close to anterolateral margins of ocellar triangle; tergite VIII posterior margin on lateral thirds with well-developed comb *Megalurothrips usitatus*.

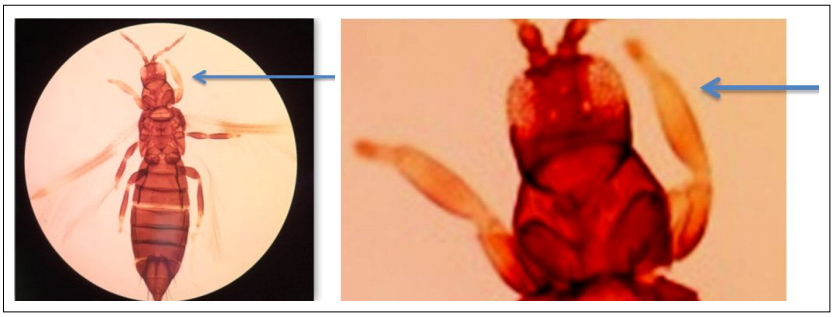


**Plate 1:** Fore tibiae yellow.

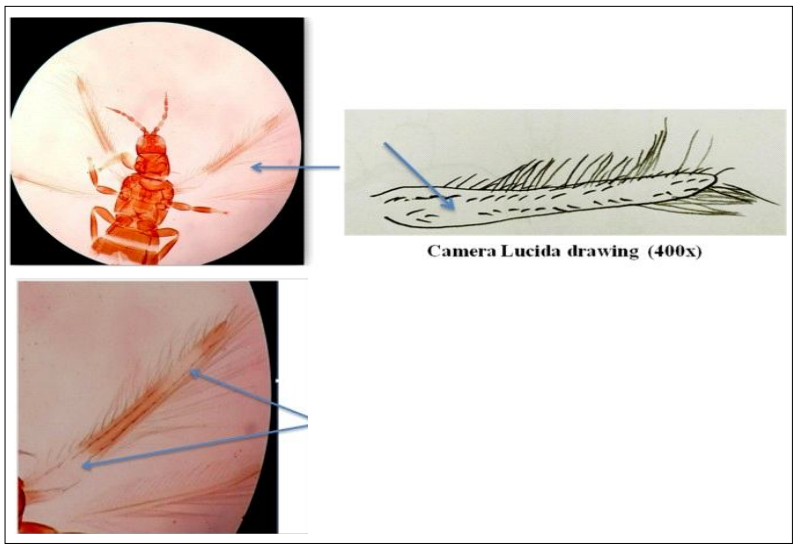



**Plate 2:** Fore wings brown with basal quarter pale and an extensive pale area sub- apically.

***Megalurothrips usitatus* (Bagnall)**

Both sexes fully winged. Body dark brown, tarsi, apices of mid and hind tibiae, also most of fore tibiae yellow; hind tibiae with 2 stout dark apical setae (Plate 1). Fore wings brown with basal quarter pale and an extensive pale area sub-apically (Plate 2). Antennal segments I-II brownish yellow, III yellow, IV and sometimes V yellow at base; fore wing light brown, pale sub-basally and with sub-apical pale band (Plate 3). Antennae 8-segmented, I with pair of dorso-apical setae; III-IV with constricted apical neck, sensorium forked, VIII almost twice as long as VII (Plate 4). Head conspicuously transversely striate/reticulate at posterior (Plate 5), ocellar setae III long, arising just inside triangle; postocular setae not long (Plate 6). Pronotum sometimes 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 7). Mesonotum with transverse reticulation, lateral setae not long (Plate 8). Metanotum reticulate medially, median setae long, at anterior margin, campaniform sensilla present. Mesosternal furca with spinula, metafurca without spinula. Tarsi all 2-segmented. Fore wing first vein with long row of setae before distinct sub-apical gap followed by 2 setae; second vein with complete row of setae; postero-marginal cilia wavy. Abdominal tergites II-VIII with no sculpture medially but lateral thirds with sub-parallel lines (Plate 9), median setae small (Plate 10); VIII with postero-marginal comb of small microtrichia laterally (Plate 11), discal area antero-mesead of spiracle with 2 or more rows of strong microtrichia; tergite X with incomplete longitudinal split. Sternites without discal setae, three pairs of long marginal setae, setal pair S1 on VII arise in front of margin (Plate 12). Male similar to female but smaller and paler, pronotum usually yellow; legs sometimes almost yellow; tergite IX with pair of short stout setae posterolaterally; sternites with no pore plates (Plate 13).

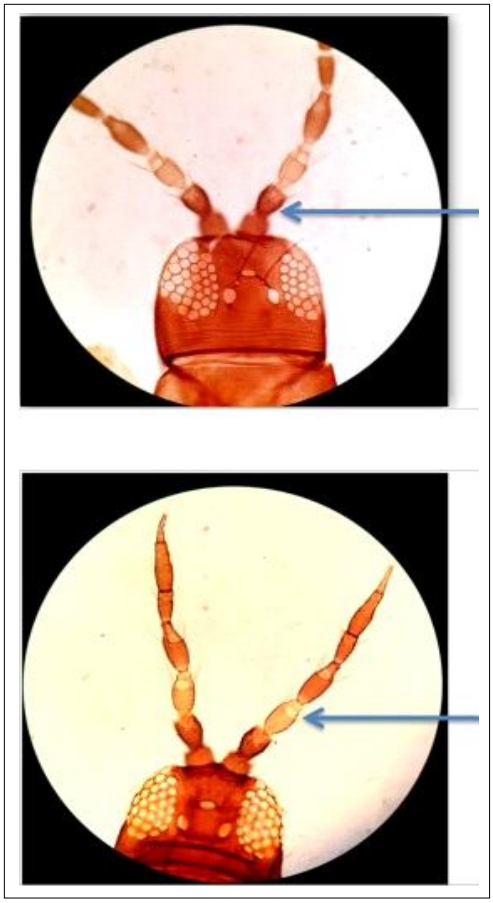


**Plate 3:** Antennal segments I-II brownish yellow, III yellow, IV and sometimes V yellow at base.

The species identification of thrips was confirmed by sending specimens to ICAR-NBAIR (Germplasm Collection and Characterization). Dr. Laurence Mound, an honorary research fellow at CSIRO in Australia, provided further confirmation. Precise identification, vector specificity plays major role in management of vector transmitted diseases. Thrips species level identification is mostly based on morphological characters, for instance color; antennae

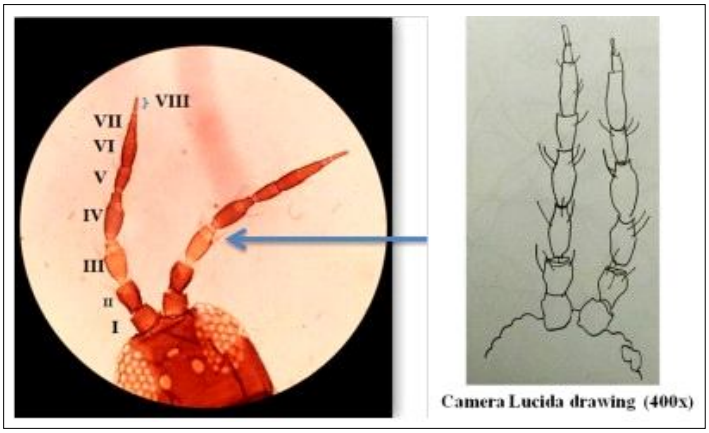



**Plate 4:** Antennae 8-segmented, I with pair of dorso-apical setae; III–IV with constricted apical neck, sensorium forked, VIII almost twice as long as VII.

segment arrangement, body structural design, chaetotaxy, ocelli, thoracic features *etc*. On the other hand, the morphology-based detection from time to time could be difficult due to their minute size, sexual dimorphism, high degree of similarity in various developmental stages and polymorphism (in color, wing development, body size *etc*.), co-existence of multiple species on the same plant. The problem of identification can be aggravated when their appearances are varied in many ways within the species (Mound and Kibby 1998, Natasa and Tradan 2012). *Megalurothrips sp*. is an economic importance pest and generally they attack various Fabaceae crop such as *Glycine*, *Arachis* and *Vigna* (Palmer, 1987; Sani and Umar, 2017; Tang *et al.,* 2015; Zafirah and Azidah, 2018). Pest status of *M. usitatus* on blackgram in India is not yet reported and available literature in this aspect is also

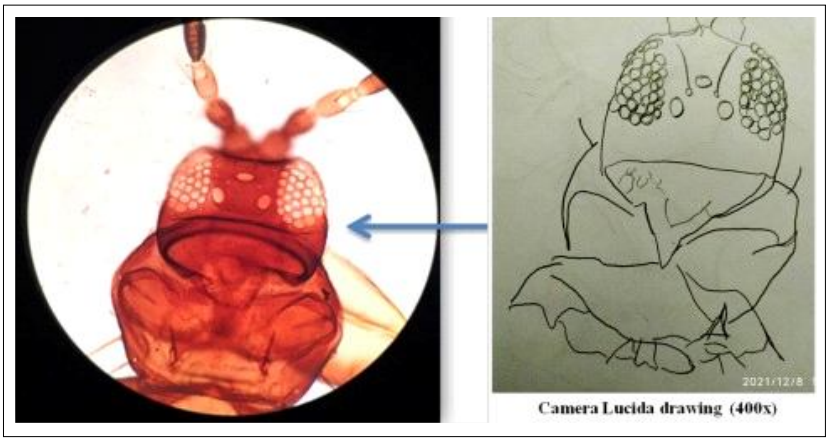



**Plate 5:** Head conspicuously transversely striate/reticulate at posterior.

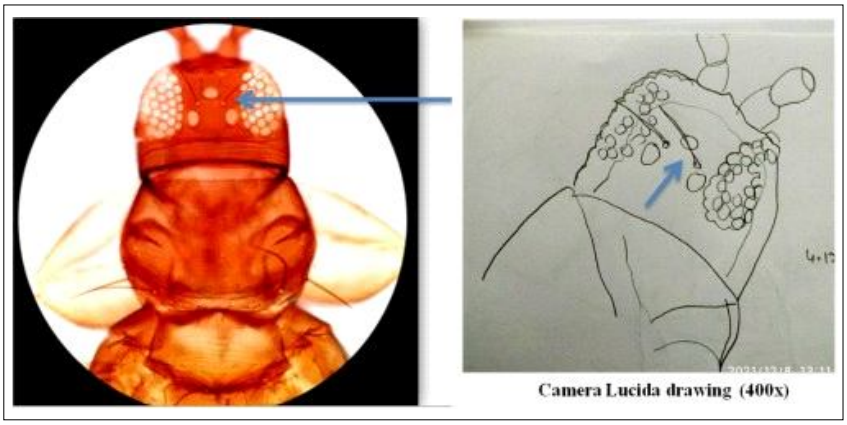



**Plate 6:** Ocellar setae III long, arising just inside triangle; post ocular setae not long.

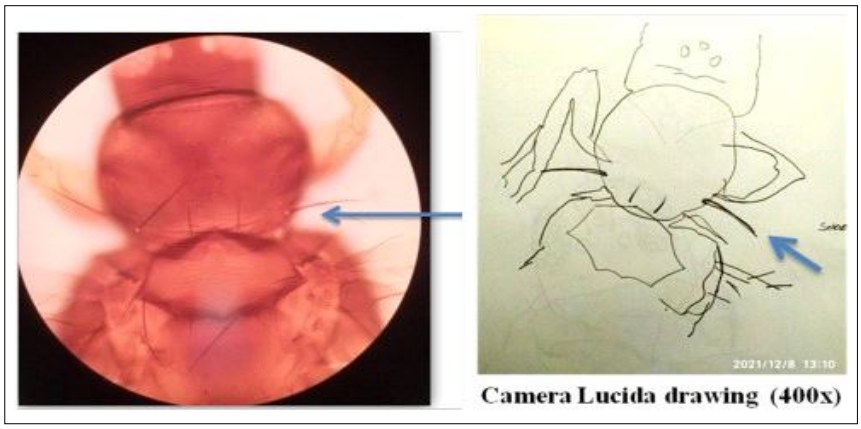



**Plate 7:** Pronotum-two pairs of long posteroangular setae, outer longer than inner.

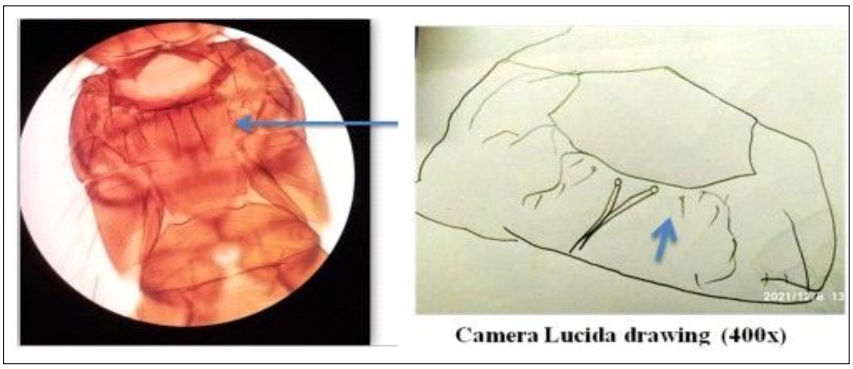



**Plate 8:** Mesonotum with transverse reticulation, lateral setae not long.

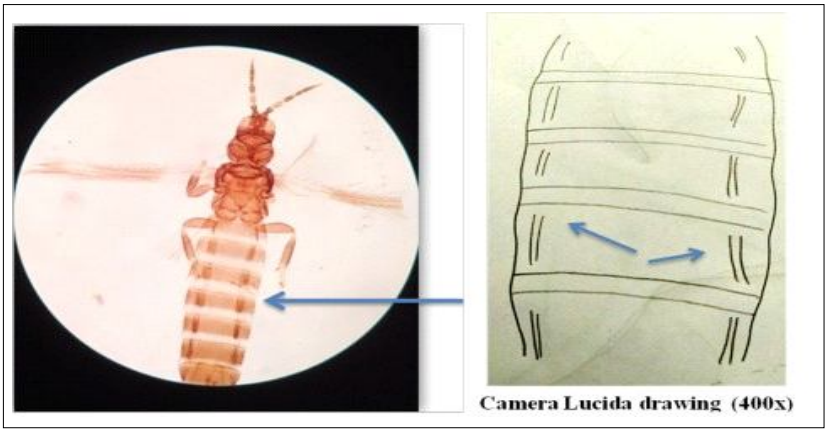



**Plate 9:** Abdominal tergites II–VIII with no sculpture medially but lateral thirds with sub-parallel lines.

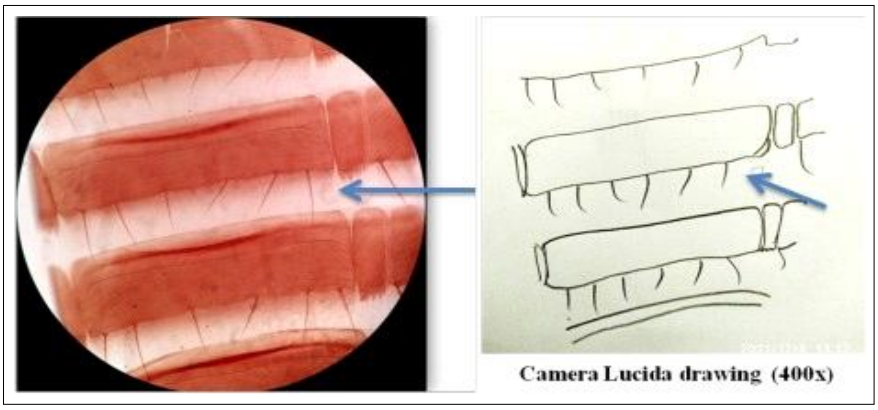



**Plate 10:** Abdominal tergites II–VIII, median setae small.

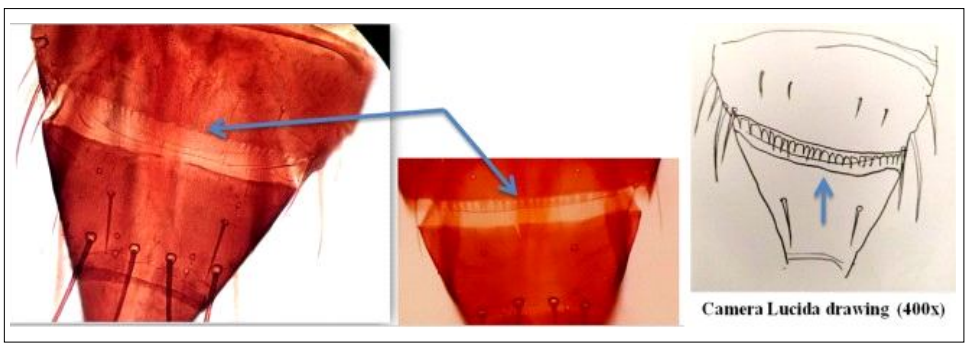



**Plate 11:** Tergite VIII with postero-marginal comb of small microtrichia laterally.

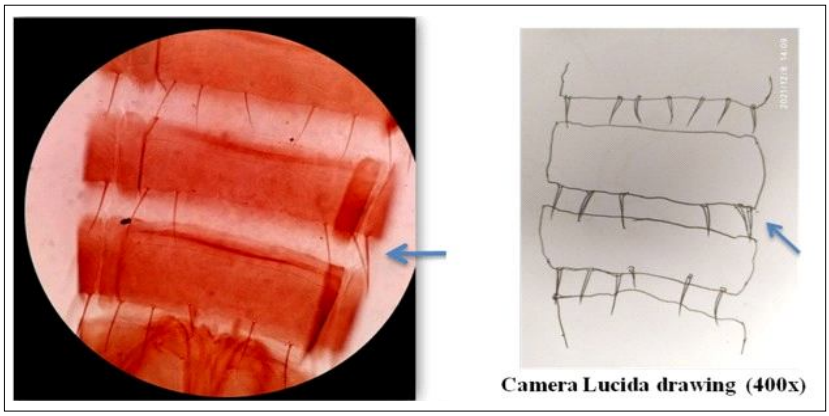



**Plate 12:** Sternites without discal setae, three pairs of long marginal setae, setal pair S1 on VII arise in front of margin.

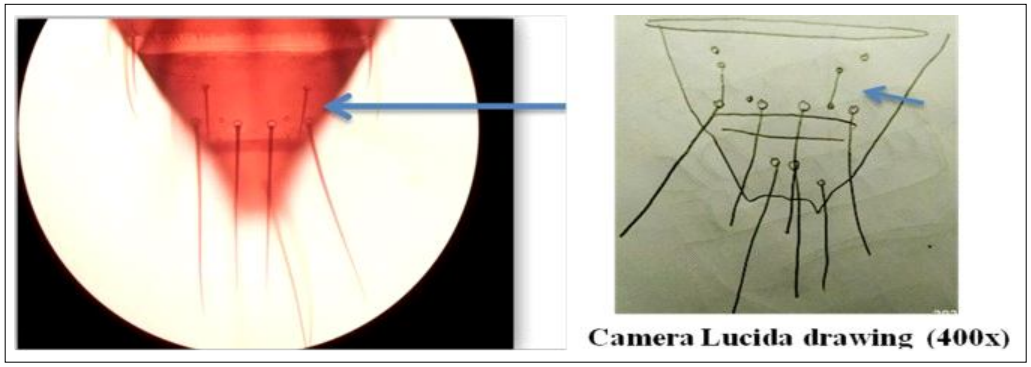
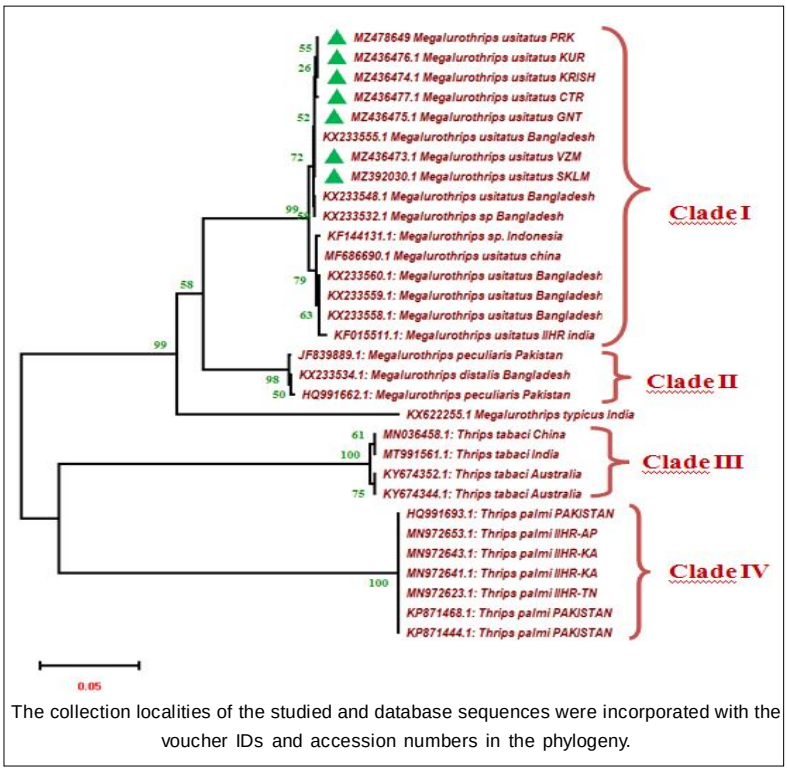



**Plate 13:** Male-tergite IX with pair of short stout setae posterolaterally.




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

**Fig 1:** Neibhour Joining phylogenic tree for *M. usitatus*, (bootstrap replicates 1000).

scanty. Hence, the present study concentrated on identification and molecular characterization of *M. usitatus* to know the species status. Following the identification of every specimen, the percentage of species composition was calculated and is shown in (Table 1). Districts Srikakulam, Vizianagaram, Krishna, Guntur, Prakasam, Kurnool and Chittoor districts showed 31.43, 28.14, 35.58, 47.31, 20.30, 21.14, 28.81 mean per cent of *M. usitatus,* respectively. Pittalavanipalem mandal of Guntur district and R. Amudalavalasa mandal of Srikakulam district have recorded with highest mean per cent of *M. usitatus* among the all other mandals *i.e.* 66.67 and 56.3, respectively.

**Identification and confirmation of *M. usitatus* using DNA barcodes**

Molecular markers that are developed with mitochondrial DNA (mtDNA) are useful to discriminate closely related species. The mitochondrial COI sequence was validated to identify and classify thrips species and to understand the phylogenetic relationship. In the present study, amplification was observed at 655 bp for 70 individual specimens using mtCOI marker (Plate 14-15 of supplementary information). Present results are in accordance with Chakraborthy *et al.* (2019) who studied 43 *Scirtothrips* spp specimens across India and identified using SEM utilizing the morphometric key and were further confirmed using mtCOI markers based on species specific amplification (648bp) and contributed six novel barcode sequences of three *Scirtothrips* species from India. Another finding by Rabeena *et al.* (2020) on the utilization of mtCOI based universal primer in identification of *F. schultzei* collected from tomato growing hot spot regions of Tamil Nadu and Karnataka. Specific amplicon size of ~638 bp was detected and the samples were sequenced and deposited in NCBI. Further Singha *et al.* (2019) identified 11 specimens of *Frankliniella occidentalis* (Pergande) collected from Karnataka, India and studied using *mtCOI* universal primer and generated four sequences specific to the collected specimens and submitted in NCBI.

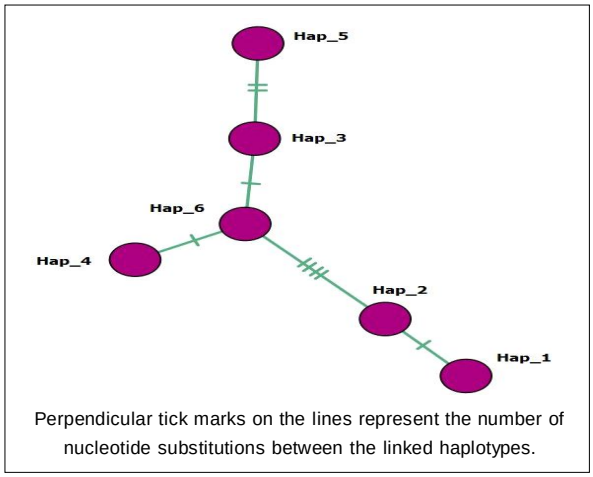



**Fig 2:** Haplotype network analysis of *M. usitatus* using mtCOI sequences of present study and presumptive conspecifics from Genbank.

**Studies on sequencing and homology**

The sequences of *Megalurothrips usitatus* found in this study exhibited 99-100% similarity with the NCBI sequences. The accessions from Bangladesh, Pakistan and India exhibited 100% similarity with the sequences of the current study, where as 99 per cent similarity was observed with accessions from India, Bangladesh, Pakistan, China, Indonesia. The generated DNA sequences of the species were annotated and submitted to the global database (GenBank) to acquire the unique accession numbers. List of samples, geographic location along with accession numbers presented in the (Table 1). With sufficient bootstrap support and posterior probability, the calculated Neighbor Joining phylogeny (30 sequences) has showed cohesive grouping of the generated *M. usitatus* sequences. All of the *M. usitatus* sequences of this investigation (MZ392030, MZ436473 to MZ436477, MZ478649) were clearly closely clustered

**Annexure II:** Haplotype data (*M. usitatus*).

| Haplotype | GENBANK accession numbers | Source of sequence used | Geographic location of sequences |
|---|---|---|---|
| Hap_1 | 14 [MZ392030.1-1 MZ392030.1-2 MZ436473.1-1 MZ436473.1-2 MZ436475.1-1 MZ436475.1-2 MZ436477.1-1 MZ436477.1-2 KX233555.1-1 KX233555.1-2 KX233548.1-1 KX233548.1-2 KX233532.1-1 KX233532.1-2] | Present study and NCBI | Srikakulam, Vizianagaram, Guntur, Chitturu, of present study and Bangladesh from NCBI respectively |
| Hap_2 | 6 [MZ436474.1-1 MZ436474.1-2 MZ478649-1 MZ478649-2 MZ436476.1-1 MZ436476.1-2] | Present study | Krishna, Prakasam, Kurnool |
| Hap_3 | 6 [KX233560.1:4-657-1 KX233560.1:4-657-2 KX233559.1:4-657-1 KX233559.1:4-657-2 KX233558.1:4-657-1 KX233558.1:4-657-2] | NCBI | Bangladesh |
| Hap_4 | 2 [KF144131.1:10-663-1 KF144131.1:10-663-2] | NCBI | Indonesia |
| Hap_5 | 2 [KF015511.1:2-655-1 KF015511.1:2-655-2] | NCBI | India |
| Hap_6 | 2 [MF686690.1-1 MF686690.1-2] | NCBI | China |

with other *M. usitatus* derived from Bangladesh under Clade I (Fig 1). The *M. usitatus* group was generated by the cohesive clustering of the remaining sequences of *M. usitatus* from several countries, including China, Indonesia, Bangladesh and one sequence from IIHR in Bangalore, India, under clade I. Furthermore, this phylogenic tree showed that *Megalurothrips distalis* (Karny) and *Megalurothrips pecularis* (Bagnall) split off as distinct branches in clade II and separated as a separate cluster from clade I. Phylogenic tree analysis made it abundantly evident that *M. usitatus* is distantly connected to *Megalurothrips typicus* (Bagnall) and closely

**Table 1:** Distribution of *M. usitatus* population (in per cent) on blackgram in different geographic locations of Andhra Pradesh, India based on morphological identification and GenBanK accession numbers allotted to *M. usitatus* samples characterized in this study.

| District | Mandal | *M. usitatus* | Sample code | GenBank Acc. number |
|---|---|---|---|---|
| Srikakulam | Etcherla | 27.78 | LRTM 8 | MZ392030 |
| | Ponduru | 21.05 | | |
| | Sigadam | 33.33 | | |
| | R.Amudalavalasa | 56.25 | | |
| | Rajam | 18.75 | | |
| | Total per cent mean | 31.43 | | |
| Vizianagaram | Gantyada | 38.89 | | |
| | Bondapalli | 33.33 | | |
| | Mentada | 19.05 | LRTM 9 | MZ436473 |
| | Dattirajeru | 29.41 | | |
| | Gajapathingaram | 20.00 | | |
| | Total per cent mean | 28.14 | | |
| Krishna | Avanigadda | 50.00 | LRTM 10 | MZ436474 |
| | Challapalli | 47.06 | | |
| | Mopidevi | 26.67 | | |
| | Pamarru | 37.50 | | |
| | Movva | 16.67 | | |
| | Total per cent mean | 35.58 | | |
| Guntur | Pittalavanipalem | 66.67 | | |
| | Amarthalur | 31.25 | | |
| | Ponnuru | 35.29 | | |
| | Chebrolu | 53.33 | | |
| | Tadikonda | 50.00 | LRTM 11 | MZ436475 |
| | Total per cent mean | 47.31 | | |
| Prakasam | Vetapalem | 13.33 | | |
| | Chirala | 21.05 | | |
| | Ongole | 21.05 | LRTM 12 | MZ478649 |
| | Chinaganjam | 16.67 | | |
| | Naguluppalapadu | 29.41 | | |
| | Total per cent mean | 20.30 | | |
| Kurnool | Gospadu | 11.8 | | |
| | Panyam | 20.00 | | |
| | Bandiatmakuru | 13.33 | | |
| | Banaganapalle | 27.27 | LRTM 13 | MZ436476 |
| | Gadivemula | 33.33 | | |
| | Total per cent mean | 21.1 | | |
| Chittoor | Gudipala | 6.67 | | |
| | Thavanampalle | 26.09 | | |
| | Palasamudram | 33.33 | LRTM 14 | MZ436477 |
| | Ganghadharnellore | 31.82 | | |
| | Airala | 46.15 | | |
| | Total per cent mean | 28.81 | | |
| Andhra Pradesh | Total per cent mean | 30.05 | | |

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

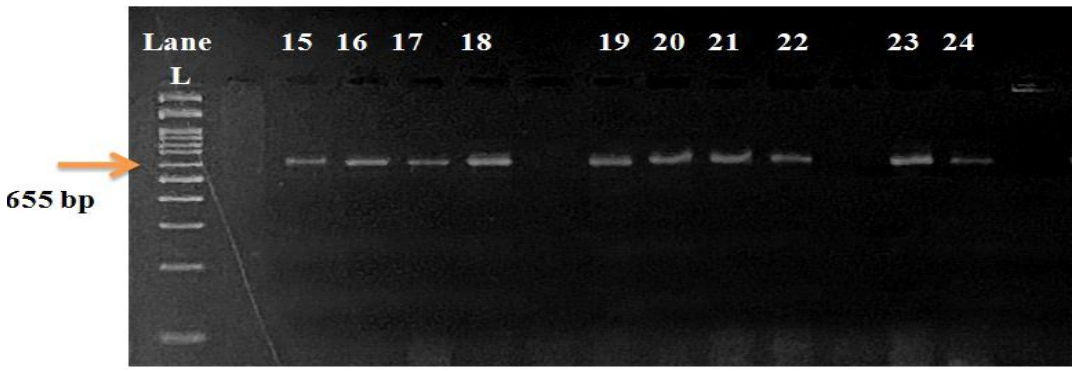
**Table 2:** Genetic diversity and Tajima's D evaluated for detected species of thrips.

| 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) |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. usitatus* | 32 | 9 | 9 | 0.00509 | 9 | 0.00055 | 6 | 0.750 | 0.056 | 1.31414 Not significant, P>0.10 |

related to *M. pecularis* and *M. distalis*. Using phylogenetic analysis, the NCBI sequences of *M. typicus* and the mtCOI sequences of *T. tabaci* and *T. palmi*, were also used to examine species-level diversity. Clade III emerged as the *T. tabaci* group, containing sequences from China, India and Australia, three distinct nations. Clade IV of the *T. palmi* group originated in southern India and Pakistan. Similar findings of distinct species-wise groups of *T. palmi*, *T. tabaci*, *F. occidentalis*, *S. dorsalis* and an unclassified group were also reported by Kadirivel *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*. Findings of the present study were in line with Zafirah *et al.* (2020) developed a phylogenic tree using the sequences of *M. uistatus* and *M. typicus*, *M. distalis* based on COI gene marker and reported that *M. usitatus* group formed as clade I where as *M. usitatus* Lineage II, *M. distalis*, *M. typicus* were formed as clade II. Typically *M. usitatus* Linaeage II was formed under subclade II and also reported that the inter specific distances between both *M. usitatus* Lineage I and Lineage II ranged from 8.78 to 9.63 per cent, suggesting the presence of cryptic and non-monophyly lineages between two morphoforms of *M. usitatus* in Peninsular Malaysia.

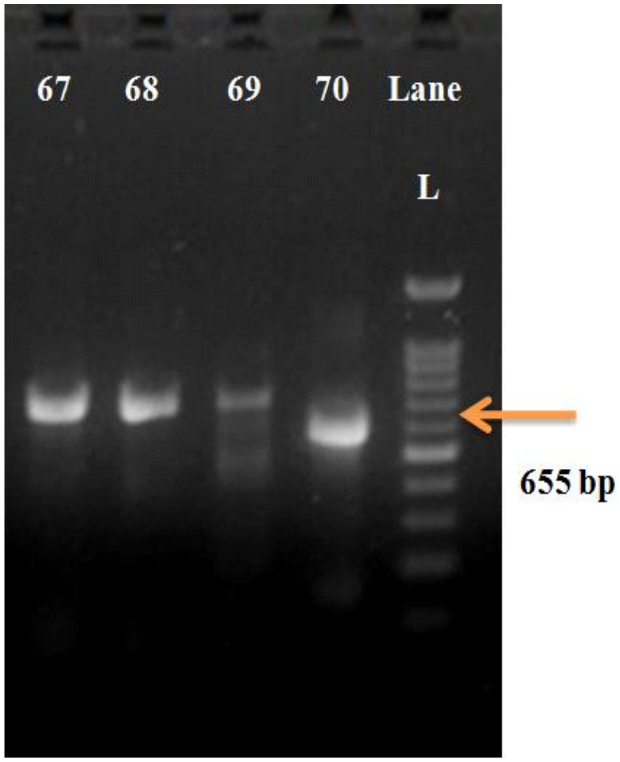
**Genetic divergence and haplotype analysis**

*mtCOI* sequences of this study (07 sequences of *M. usitatus* from Andhra Pradesh, Annexure II), nine GenBank-sourced sequences of three different geographic regions (India, Indonesia, Bangladesh and China) combined has revealed 06 haplotypes which were clustered in a network according to genetic diversity. For the current haplotype analysis, *mtCOI* sequence containing 657 nucleotides was chosen and 628 nucleotides total-excluding gaps or missing data sites-were employed. Data revealed that Srikakulam, Vizianagaram, Guntur, Chittoor, of present study and sequences from Bangladesh were formed into Hap_1 where as other mtCOI sequences from Krishna, Prakasam and Kurnool of present study were formed into Hap_2. Six sequences from Bangladesh were formed into Hap_3. Other sequences from Indonesia was formed into Hap_4. Sequences from India and China were formed into Hap_5 and Hap_6, respectively (Fig 2). This haplotype network was in support with our previously constructed neighbor joining and maximum likelihood trees of present study. These results were supported by Tyagi *et al.* (2017) who analysed 85 *T. palmi mtCOI* sequences from India, resulted in eight haplotypes (H9-H10, H126-H131) forming three distinct clades in both the NJ and BA tree. These three clades were also represented by three MOTUs (*T. palmi* Ia1, *T. plami* IIa1 and *T. palmi* Ib2) in ABGD, GMYC and bPTP analysis. Further analysis stated that a total of 32 sequences were used for haplotype network and these sequences were grouped into six haplotypes. A maximum of nine segregating sites were observed with nucleotide diversity ($\pi$) 0.00509 and standard de*via*tion of nucleotide diversity ($\pi$) *i.e.,* 0.00055. Haplotype diversity was recorded




Lane L = 100 bp Ladder; Lane 15, 16 = Mentada; Lane 17, 18 = Dattirajeru; Lane 19, 20 = Gajapatinagaram; Lane 21, 22 = Avanigadda, Lane 23, 24 = Challapli; Lane 25, 26 = Mopidevi; Lane 27, 28 = Pamarru.

**Plate 14:** Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI marker (655 bp).




Lane L= 100 bp Ladder; Lane 67, 68 = Gangadharnellore; Lane 69, 70 = Airala.

**Plate 15:** Electrophoretic separation of PCR amplification products of *M. usitatus* with mtCOI marker (655 bp).

as 0.750 with a standard deviation of 0.056. Estimated mutations among the sequences were nine. Tajimas D statistic was 1.31414 (Not significant, P>0.10) (Table 2) which revealed the existence of low genetic polymorphism among the COI sequences of *M. usitatus.* Virus transmission ability of *M. usitatus* on blackgram is yet to be discovered in the further research programmes especially in blackgram crop where bud necrosis is often causing huge losses.

## CONCLUSION

In conclusion, species identification using morphological features has some significant limitations as the species might have minimal or no phenotypic changes but have high genetic variability. Even though morphological identification is much cheaper economically than molecular identification as the materials and equipment used require less expenditure in it. Morphological identification usually overlooks cryptic features and the use of keys requires a high level of expertise otherwise misdiagnosis may happen. Major constraint in identification of thrips is that their larval stages as they cannot be identified with most available keys designed so far and they exhibit fewer characters of diagnostic value when compared to adults. However, in a comprehensive manner both molecular and morphological identification techniques need to be used to clearly identify the species of the specimens. Use of genetic markers, such as mtDNA, ITS2, mtCOIII etc., at present, a valuable addition or alternative to the classical methods of species identification. especially when the morphological approach is difficult or even impossible. Sequencing variation in the mitochondrial cytochrome c oxidase I (COI) region has been proven to be useful for the identification of species of many groups of insect pests.

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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.

## REFERENCES

Ananthakrishnan, T.N. (1980). On some aspects of thrips galls. Bulletin de la Societe Botanique de France. *Actualites Botaniques*. **127(1):** 31-34.

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

Cheol, M.H., Kwon, C.I., Rock, I.J., Goh Rae, B., Hyang, K.D. *et al.* (2006). Seasonal occurrence and damage by thrips on open red pepper in Jeonbuk Province. *Korean Journal of Applied Entomology*. **45:** 9-13.

Cluever, J.D., Smith, H.A. (2017). A photo-based key of thrips (Thysanoptera) associated with horticultural crops in Florida. *Florida Entomologist*. **100(2):** 454-467.

Hebert, P.D.N., Cywinska, A., Ball, S.L., Waard, D.J.R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London, Biological Sciences*. **270:** 313-321.

Hebert, P., Ratnasingha, S., deWaard, J.R. (2003b). Barcoding animal life: Cytochrome C oxidase subunit 1 divergences among closely related species. Proceedngs of the Royal Society of London B (Supplement) online. 15.05.03.

Hoddle, M.S., Mound, L.A. (2003). The genus *Scirtothrips* in Australia (Insecta, Thysanoptera, Thripidae). *Zootaxa.* **268:** 1-40.

Hoy, A.M. (2003). Insect molecular genetics: An introduction to principles and applications. Academic Press, New York. pp: 544.

http://entomology.ifas.uf.edu.

http://popart.otago.ac.nz.

http://www.ncbi.nlm.nih.gov/gorf/gorf.html.

https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Mound, L.A., Kibby, G. (1998). Thysanoptera. An Identification Guide 2nd edn. CAB International, Wallingford, U.K. 70.

Mound, L.A., Yongfoo, N.G. (2009). An illustrated key to the genera of Thripinae (Thysanoptera) from South East Asia. *Zootaxa*. **2265:** 27-47.

Natasa, M., Tradan, S. (2012). Traditional and modern methods for the identification of thrips (Thysanoptera) species. *J. Pest Sci.* **85:** 179-190.

Palmer, J.M. (1987). *Megalurothrips* in the flowers of tropical legumes: A morphometric study. In: Holman J, Pelikan J, Dixon AFG, Weismann L (Eds.), Population Structure, Genetics and Taxonomy of Aphids and Thysanoptera. SPB Academic Publishing, Amsterdam, The Netherlands. pp: 480-495.

Rabeena, I., Chinnaiah, C., Karthikeyan, G., Usharani, T.R., Balakrishnan, N., Kennedy, J.S., Rajabaskar, D. (2020). An integrated approach for identification of *Frankliniella schultzei* (trybom). *Indian J. of Entomol*. **82(1):** 179-182.

Sambrook, J., Fritsch, E.R., Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY, U.S.A. pp: 2.

Sani, I., Umar, K.M. (2017). Biology and management of legume flower thrips (*Megalurothrips sjostedti*) (Thysanoptera: Thripidae) a major insect pest of cowpea: A review. *Ann. Exp. Biol*. **5(1):** 14-17.

Simon, C. (1991). Molecular systematics at the species boundary: Exploiting conserved and variable regions of the mitochondrial genome of animals *via* direct sequencing from amplified DNA. In Hewitt GM, Johnston A W, Young J PW (Eds.) Molecular techniques in taxonomy. *Springer-Verlag, Berlin*. pp: 33-71.

Singha, D., Kumar, V.V., Chakraborty, R., Kundu, S., Hosamani, A., Kumar, V., Tyagi, K. (2019). Molecular footprint of *Frankliniella occidentalis* from India: A vector of Tospoviruses. *Mitochondrial DNA part B*. **4(1):** 39-42.

Sunnucks, P. and Hales, D.F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). *Molecular Biology and Evolution*. **13:** 510-524.

Tang, L.D., Yan, K.L., Fu B.L., Wu J.H., Liu, K., Lu, Y.Y. (2015). The life table parameters of *Megalurothrips usitatus* (Thysanoptera: Thripidae) on four leguminous crops. *Fla Entomol*. **98(2):** 620-625.

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

Virenberg, G. (1995). International movement detection and quarantine of Thysanoptera pests. Thrips biology and Managemnet (ed. by Parker BL, Skinner, M Lewis, T) Plenum Press New york. pp: 119-122.

Zafirah, Z., Azidah, A.A. (2018). Diversity and population of thrips species on legumes with special reference to *Megalurothrips usitatus*. *Sains Malays*. **47(3):** 433-439.

Zafiraha, Z., Lowb, V.L., Azidaha, A.A. (2020). Non-monophyly and cryptic lineages between two morphoforms of *Megalurothrips usitatus* (Thysanoptera: Thripidae) in Peninsular Malaysia: Insights from morphological and molecular data. *Journal of Asia-Pacific Entomology*. **23:** 554-558.