

Morpho-molecular Characterization and Phylogeny of Thrips Species from Crop Ecosystems of Tarai Region, Uttarakhand

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Research Article

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Abstract

Thrips are agriculturally significant insects that cause direct damage and transmit plant viruses; however, their minute size and morphological similarities complicate accurate identification. This study integrated morphological and molecular approaches to characterise thrips species from major crop ecosystems in the Tarai region of Uttarakhand, India, during 2020–2022. A total of eight thrips species were identified using taxonomic keys and confirmed by mitochondrial COI gene sequencing: *Haplothrips* (*Haplothrips*) *andresi*, *H. (H.) ganglbaueri*, *Megalurothrips usitatus*, *Thrips coloratus*, *Thrips flavus*, *Thrips hawaiiensis*, *Thrips palmi*, and *Thrips tabaci*. Phylogenetic analysis using the neighbour-joining method revealed clear genus-level segregation, with *Haplothrips* and *Megalurothrips* forming distinct clades separated from the genus *Thrips*. Close evolutionary affinities were observed between *T. palmi* and *T. hawaiiensis*, and between *T. tabaci* and *T. coloratus*. Populations of *M. usitatus* from different host plants showed high genetic similarity (COI divergence < 0.5%), indicating potential wide host adaptability. Moderate intraspecific variation was detected among *T. flavus* isolates, suggesting possible host-associated differentiation. The generated COI sequences were deposited in GenBank, enriching the molecular database for Indian Thysanoptera. This study demonstrates that integrating morphological identification with COI-based molecular characterisation provides a reliable framework for species delimitation and evolutionary understanding, which is essential for effective pest surveillance and management strategies.

Introduction

Thysanoptera, commonly known as thrips, is a diverse and economically significant order of insects, comprising more than 6,000 described species worldwide. The order is divided into two suborders: Terebrantia and Tubulifera (Grimaldi and Engel 2005; Buckman et al. 2013; Johnson et al. 2018). In India, the thrips fauna is notably rich, with 816 recorded species belonging to 265 genera, including 368 species of Terebrantia and 448 species of Tubulifera (Tyagi and Kumar 2016; Tyagi et al. 2024). Among these, the suborder Terebrantia, particularly the family Thripidae, comprises the majority of agriculturally important species in the country (ThripsWiki 2023; Tyagi et al. 2024).

Thrips exhibit remarkable ecological adaptability and occupy a wide range of ecological niches. Many species are recognised as serious agricultural pests, causing direct damage through feeding that results in leaf scarring, silvering, curling, stunted growth, and gall formation. Several species also act as vectors of economically important plant viruses, including orthospoviruses (Ananthakrishnan 1978; Riley et al. 2011). Conversely, certain thrips species provide beneficial ecological services as pollinators and biological control agents (Hoddle et al. 2004).

Despite their ecological and economic importance, accurately identifying thrips remains challenging due to their minute size, cryptic behaviour, and morphological similarities among closely related species. Conventional taxonomy, which relies solely on morphology, depends on subtle diagnostic characters that require specialised expertise and may be influenced by intraspecific variation or the condition of the

specimen (Mound and Kibby 1998; Mound and zur Strassen 2001). Furthermore, several economically significant thrips species have been revealed as cryptic species complexes, including *Thrips tabaci* (Brunner et al. 2004; Farkas et al. 2019), *Frankliniella schultzei*, and *Thrips palmi* (Tyagi et al. 2017), where morphologically indistinguishable lineages differ in reproductive mode, host preference, and virus transmission efficiency.

In recent years, molecular approaches have become invaluable tools for supporting species identification and resolving taxonomic ambiguities in thrips. Among these, analysis of the mitochondrial cytochrome c oxidase subunit I (COI) gene has been widely employed in molecular characterisation and phylogenetic studies of insect taxa (Brunner et al. 2002; Hebert et al. 2003). DNA barcoding using the COI gene typically exhibits low intraspecific divergence (generally less than 2%) and higher interspecific divergence, enabling reliable species delimitation (Hebert et al. 2003; Vono et al. 2020). The integration of morphological and molecular data therefore provides a more robust framework for species characterisation and for understanding evolutionary relationships among thrips species.

In India, comprehensive DNA barcoding efforts have significantly advanced thrips taxonomy. Tyagi et al. (2017) analysed 370 COI sequences representing 89 morphospecies and identified 107 molecular operational taxonomic units (MOTUs), revealing cryptic diversity in several vector species, including *F. schultzei* and *T. palmi*. Subsequent studies have documented the spread of invasive species such as *Thrips parvispinus* (Tyagi et al. 2015; Sharma et al. 2024) and reported new distribution records from various states.

The Himalayan state of Uttarakhand, particularly the Tarai region, boasts diverse agricultural ecosystems and favourable climatic conditions that support the abundance and distribution of thrips. Recent surveys have recorded several terebrantian and tubuliferan thrips species from Uttarakhand (Saran et al. 2025; 2027). However, information regarding the morpho-molecular characterisation and phylogenetic relationships of thrips species from this region remains limited, especially for those associated with major crop ecosystems. Although several thrips species have been reported from Uttarakhand, comprehensive studies integrating both morphological and molecular evidence remain insufficient.

Therefore, the present study was undertaken to characterise thrips species associated with the major crop ecosystems of the Tarai region of Uttarakhand, using both morphological and molecular approaches, and to elucidate their phylogenetic relationships.

Materials and Methods

1. Collection of Thrips Specimens

Thrips specimens were collected from major crop ecosystems in the Tarai region of Uttarakhand, India, between 2020 and 2022. Surveys were conducted in agricultural fields in the Udham Singh Nagar and Nainital districts across various cropping seasons to obtain representative populations from economically important crops.

Specimens were collected using the standard beating method (Tyagi et al. 2017). Infested leaves, flowers, and tender plant parts were gently beaten over a white plastic tray (40 × 30 cm) to dislodge the insects. Adult thrips were collected using a fine camel hair brush (size 000) and transferred into 1.5 ml microcentrifuge tubes. For morphological studies, specimens were preserved in AGA solution (60% ethanol, glycerin, and glacial acetic acid in a 10:1:1 ratio) (Sharma et al. 2024). For molecular analyses, specimens were preserved separately in 95–100% ethanol and stored at – 20°C until DNA extraction (Farkas et al. 2019; Vono et al. 2020). Each sample was labelled with collection details: host plant, location, GPS coordinates, and date of collection.

2. Morphological Identification

Permanent slide mounts of thrips specimens were prepared following the method described by Tyagi and Kumar (2016), with minor modifications. Specimens preserved in AGA solution were washed in distilled water and then cleared in 10% NaOH for approximately 30 minutes (or until sufficiently transparent), followed by two rinses in distilled water. Dehydration was performed through a graded ethanol series (50%, 70%, 90%, and absolute ethanol) for five minutes each. The specimens were then cleared in clove oil for 5–10 minutes and mounted in Canada balsam on clean glass slides. Appendages (antennae, legs, wings) were carefully arranged before applying coverslips, and slides were cured at 45°C for two weeks (Tyagi et al. 2017).

Mounted specimens were examined under a compound microscope (Leica DM 1000) at magnifications ranging from 100× to 1000×. Identification to species level was carried out using available taxonomic keys and original descriptions (Ananthakrishnan and Sen 1980; Bhatti 1980; Palmer 1992; Tyagi and Kumar 2016). Online taxonomic resources, including ThripsWiki (2023), were consulted to confirm species identity. Representative voucher specimens were authenticated by Dr Kaomud Tyagi, Scientist C, Centre for DNA and Systematics, Zoological Survey of India, Kolkata. All voucher specimens have been deposited in the National Zoological Collections (NZC) at the Zoological Survey of India.

1. 3. Molecular Characterisation

2. 3.1 DNA Extraction

Genomic DNA was extracted from individual adult thrips specimens using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), following the manufacturer's protocol with modifications for small-bodied insects (Tyagi et al. 2017). Individual specimens were placed in 1.5 ml microcentrifuge tubes and homogenised in 180 µl of ATL buffer using a sterile micropestle. Then, 20 µl of Proteinase K was added, and the mixture was incubated at 56°C for 1.5 hours (or overnight for difficult samples) with occasional vortexing. Subsequently, 200 µl of AL buffer and 200 µl of molecular-grade ethanol (96–100%) were added, and the lysate was transferred to a DNeasy Mini spin column. The column was washed with 500 µl of AW1 buffer followed by 500 µl of AW2 buffer, with centrifugation at 8000 rpm (approximately 6000 × g) for 1 minute between washes. Genomic DNA was finally eluted in 200 µl of AE buffer (or 50 µl for higher concentration). DNA quality and concentration were assessed by 1% agarose gel electrophoresis and spectrophotometrically using a NanoDrop™ (Thermo Fisher Scientific) by measuring

the A260/A280 ratio. Extracted DNA was stored at – 20°C until PCR amplification (Sharma et al. 2024; Farkas et al. 2019).

3.2 PCR Amplification of the COI Gene

A fragment (~658 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers LCO1490 (5' GGTCAACAAATCATAAAGATATTGG 3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA 3') (Folmer et al. 1994; Hebert et al. 2003). PCR amplification was performed in a 25 µl reaction mixture containing: 12.5 µl of 2× PCR master mix (e.g., Takara Taq or equivalent; composition: 1.5 mM MgCl₂, 0.2 mM of each dNTP, 1.25 U Taq DNA polymerase), 1 µl each of forward and reverse primers (10 µM), 3 µl of template DNA (approximately 20–40 ng), and nuclease-free water to make up the final volume (Vono et al. 2020; Sharma et al. 2024).

Amplification was performed in a thermal cycler under the following conditions: initial denaturation at 94°C for 5 minutes; 40 cycles of denaturation at 94°C for 30 seconds, annealing at 49°C for 45 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes (Tyagi et al. 2017; Farkas et al. 2019). Negative controls (no template DNA) were included in each run.

3.3 Gel Electrophoresis and DNA Sequencing

PCR products (5 µl) were resolved on a 1.5% agarose gel pre-stained with ethidium bromide (0.5 µg/ml) in 1× TAE buffer at 80 V for 45 minutes. Gels were visualised under a UV transilluminator (Gel Doc™ system, Bio-Rad) and photographed. Successfully amplified products showing a single bright band of the expected size (~658 bp) were purified using a PCR purification kit (Qiagen) following the manufacturer's instructions. Purified amplicons were subjected to bidirectional Sanger sequencing at a commercial facility (HiMedia Laboratories Pvt. Ltd., India) using the same PCR primers (Farkas et al. 2019; Wang et al. 2018).

4. Sequence and Phylogenetic Analyses

Forward and reverse sequence chromatograms were assembled, manually edited, and trimmed using MEGA7 software (Kumar et al. 2016). Sequence identity was preliminarily confirmed using the BLASTn tool against the NCBI non-redundant database (Altschul et al. 1990), with a threshold of ≥ 98% similarity for species-level assignment (Vono et al. 2020; Sharma et al. 2024). All generated sequences were deposited in GenBank (accession numbers are listed in Table 4).

Multiple sequence alignment was performed using ClustalW, implemented in MEGA7, with default parameters (gap opening penalty = 15, gap extension penalty = 6.66). The alignments were manually inspected and corrected where necessary.

Phylogenetic relationships among thrips species were inferred using the neighbour-joining (NJ) method based on Kimura 2-parameter (K2P) genetic distances (Kimura 1980; Saitou and Nei 1987). The robustness of the tree topology was evaluated by bootstrap analysis with 1,000 replications (Felsenstein

1985). The phylogenetic tree was visualised and annotated using MEGA7 or iTOL (Letunic and Bork 2007). Pairwise genetic distances (intraspecific and interspecific) were calculated using the K2P model to assess the barcode gap (Hebert et al. 2003; Tyagi et al. 2017).

Results and Discussion

Morphological Identification

A total of eight thrips species, belonging to two suborders (Terebrantia and Tubulifera), were identified from the major crop ecosystems of the Tarai region of Uttarakhand. These included six species from the family Thripidae (*Megalurothrips usitatus*, *Thrips coloratus*, *T. flavus*, *T. hawaiiensis*, *T. palmi*, and *T. tabaci*) and two species from the family Phlaeothripidae (*Haplothrips (Haplothrips) andresi* and *H. (H.) ganglbaueri*). The morphological diagnostic characters of each species are provided in Table 2.

Table 2
Morphological identification and species description

Sample Code	Species Identified	Key Diagnostic Characteristics
KL-1	<i>Haplothrips</i> (<i>Haplothrips</i>) <i>andresi</i> Priesner, 1931	Adult dark brown to black with yellow markings on apex of fore tibiae, antennal segment III entirely, and basal portions of antennal segments IV–VI. Major setae on head, thorax, and wing bases dark. Head longer than broad; postocular setae blunt to weakly capitate. Maxillary stylets retracted to level of postocular setae and separated by approximately 0.26–0.31 of head width. Antennae eight-segmented; antennal segment III with one sense cone. Fore tarsus without a tooth in females; males with a small fore tarsal tooth. Forewing with 10–12 duplicated cilia; sub-basal setae S1 and S2 capitate, S3 pointed. Pelta triangular; tube short.
MZ	<i>Haplothrips</i> (<i>Haplothrips</i>) <i>ganglbaueri</i> Schmutz, 1913	Body brown; fore tarsi and apices of fore tibiae yellow; mid and hind tibiae dark brown with extreme apices sometimes paler; mid and hind tarsi brown to yellowish. Postocular setae weakly capitate and much shorter than eyes. Antennae eight-segmented; segment III with one sense cone and segment IV with four sense cones. Pronotum with five pairs of major capitate setae; notopleural sutures complete. Fore tarsus in female usually without a tooth, although Australian populations may bear a minute tooth; females from India generally lack a tooth. Males usually with a small fore tarsal tooth. Forewing constricted medially, with five to nine duplicated cilia; sub-basal setae S1 and S2 short and capitate, S3 blunt and at least twice as long as S1 and S2. Pelta triangular. Male pseudovirga slender, bluntly triangular apically, with slight subapical constriction.
CUL-1; MT; CUL-1; BG-1	<i>Megalurothrips</i> <i>usitatus</i> Bagnall, 1913	Adults dark brown; tarsi yellow, with most of fore tibiae and apices of mid and hind tibiae yellow. Hind tibiae bearing two stout black apical setae. Forewings light brown with pale basal quarter and broad pale subapical band. Head distinctly transversely striate posteriorly; ocellar setae III long, arising on anterior margins of ocellar triangle or slightly outside it. Antennae eight-segmented; segments III and IV with apical neck and forked sense cones. Pronotum with two pairs of long posteroangular setae. Metanotum medially reticulate, with long median setae arising at anterior margin. Forewing first vein with long row of setae followed by a distinct subapical gap and then two distal setae. Abdominal tergites II–VIII without medial sculpture. Tergite VIII with posteromarginal comb interrupted medially and with two or more rows of stout microtrichia anteromesad of spiracles. Sternites without discal setae, each with three pairs of marginal setae; median pair on sternite VII arising anterior to posterior margin. Males smaller and paler, often whitish with yellowish pronotum, lacking pore plates on sternites.
LR-1	<i>Thrips coloratus</i> Schmutz, 1913	Adults mostly yellow; antennal segments IV–VII and abdominal tergite X brown; tergites III–VIII usually with brown median shading. Forewings weakly shaded. Head with transverse striations in ocellar area; ocellar setae III situated well outside ocellar triangle. Antennae seven-segmented. Pronotum transversely sculptured, with numerous discal setae (commonly around 20–30). Metanotum transversely striate anteriorly and with widely spaced longitudinal striae posteriorly; median setae arising behind anterior margin. Tergite VIII with complete though sometimes irregular posteromarginal comb. Sternite II with 2–4 discal setae; sternites III–VII with numerous discal

Sample Code	Species Identified	Key Diagnostic Characteristics
		setae, usually about 6–12. Male yellow; tergite VIII without comb; sternites III–VII with transverse pore plates
RD-5; BB; FN-3; MUS	<i>Thrips flavus</i> Schrank, 1776	Adults are uniformly yellow with light brown primary setae. Antennal segments IV–V are brown apically, segments VI–VII entirely brown; forewings pale. Antennae 7-segmented, segments III and IV each with a small forked sense cone. Head broader than long, with ocellar setae pair III short and situated within the ocellar triangle. Pronotum bears two pairs of long posteroangular setae. Metanotum exhibits irregular longitudinal striations that do not converge posteriorly; median setae arise behind the anterior margin. Forewing first vein with 2–3 setae on distal half; second vein with about 15 setae. Abdominal tergite II with four lateral marginal setae; tergites V–VIII with paired lateral ctenidia. Tergite VIII possesses a complete posteromarginal comb of long, slender microtrichia; median tergal setae S1 and S2 subequal in length. Pleurotergites and sternites without discal setae. Males smaller, with a complete median comb on tergite VIII and transverse pore plates on sternites III–VII.
AMR	<i>Thrips hawaiiensis</i> Morgan, 1925	Body highly variable in colour, typically bicoloured with brown abdomen and orange-yellow head and thorax, though sometimes uniformly dark brown. Legs yellowish; antennal segment III yellow. Forewings brown with paler base. Head wider than long; ocellar setae III stout, arising just outside ocellar triangle. Antennae usually seven-segmented, rarely appearing eight-segmented; segments III and IV each with short forked sense cone. Pronotum with two pairs of long posteroangular setae. Metanotum transversely striate anteriorly and with widely spaced longitudinal striae posteriorly; median setae arising at anterior margin. Forewing first vein with three distal setae; claval subterminal seta shorter than terminal seta. Abdominal tergite II with four lateral marginal setae. Tergite VIII with complete though often short and irregular posteromarginal comb of microtrichia. Sternites III–VII with numerous discal setae arranged in transverse rows. Male smaller and paler than female; tergite VIII lacking posteromarginal comb.
FN-1; BRJ	<i>Thrips palmi</i> Karny, 1925	Adults uniformly yellow with light brown major setae. Antennal segments IV–V brown distally; VI–VII entirely brown. Forewings pale. Antennae seven-segmented; segments III and IV weakly constricted apically, each with short forked sense cone. Head wider than long; ocellar setae III minute and arising just outside ocellar triangle. Postocular setae pair I slightly longer than ocellar setae III; pair II minute. Pronotum with two pairs of long posteroangular setae. Metanotum with irregular longitudinal sculpture converging posteriorly; median setae arising behind anterior margin; campaniform sensilla present. Forewing first vein with 2–3 distal setae; second vein with about 15 setae. Abdominal tergite II with four lateral marginal setae; tergites V–VIII with paired lateral ctenidia, those on tergite VIII situated posteromesad of spiracles. Tergite VIII with complete posteromarginal comb of long slender microtrichia. Pleurotergites with discal setae. Sternites III–VII with three pairs of marginal setae and without discal setae; median pair on sternite VII arising anterior to posterior margin. Male smaller than female; tergite VIII comb complete medially; sternites III–VII with narrow transverse pore plates.

Sample Code	Species Identified	Key Diagnostic Characteristics
OV-3	<i>Thrips tabaci</i> Lindeman, 1888	Adults varying from yellow to dark brown, colour influenced by developmental temperature. Ocellar pigment grey, never red. Antennal segments III and IV brown with pale basal halves; forewings pale. Head wider than long; ocellar setae III minute, arising on anterior margins or just within ocellar triangle. Antennae seven-segmented; segments III and IV slightly constricted apically, each bearing small forked sense cone. Pronotum with two pairs of posteroangular setae. Metanotum irregularly reticulate, sculpture converging posteriorly; median setae small and arising behind anterior margin; campaniform sensilla absent. Forewing first vein usually with four distal setae (range 2–6); second vein with about 15 setae. Abdominal tergite II with three lateral marginal setae. Tergite VIII with complete posteromarginal comb of long slender microtrichia. Pleurotergites and sternites without discal setae. Male small and yellow; tergite VIII with reduced comb represented by irregular microtrichia; sternites III–V with narrow transverse pore plates.

Molecular Characterisation and Sequence Analysis

Genomic DNA was successfully extracted from all representative specimens. A ~ 658 bp region of the mitochondrial COI gene was amplified and sequenced bidirectionally. BLASTn analysis against the NCBI database confirmed the morphological identifications, with query sequences showing 95–100% similarity to conspecific references (Vono et al. 2020; Sharma et al. 2024). The generated sequences were deposited in GenBank, and the assigned accession numbers are presented in Table 4, alongside host plant and collection data for each specimen.

Phylogenetic Analysis and Evolutionary Relationships

A phylogenetic tree constructed from the mtCOI sequences (Fig. 1) revealed the evolutionary relationships among the studied thrips taxa. The overall topology demonstrated clear segregation at the genus level, along with variable intra- and interspecific genetic divergence.

Table 4

Thrips species collected from different host plants, with collection details and GenBank accession numbers.

Sl. No.	Sample Code	Host Plant	Place of Collection	Date of Collection	Lat-Lon	Species of Thrips	GenBank Accession No.
1	KL-1	Karela (Bitter gourd)	Pantnagar, Udham Singh Nagar	02/05/22	29.02, 79.48	<i>Haplothrips (Haplothrips) andresi</i>	PQ144650
2	MZ	Maize	Haldwani, Nainital	07/09/21	29.21, 79.51	<i>Haplothrips (Haplothrips) ganglbaueri</i>	PQ144669
3	CUL-1	Cauliflower	Nanakmatta, Udham Singh Nagar	17/03/21	28.94, 79.81	<i>Megalurothrips usitatus</i>	PP972323
4	MT	Fenugreek	Pantnagar, Udham Singh Nagar	23/03/21	29.02, 79.48	<i>Megalurothrips usitatus</i>	PX951426
5	CW-1	Cowpea	Pantnagar, Udham Singh Nagar	03/05/22	29.02, 79.48	<i>Megalurothrips usitatus</i>	PP980482
6	BG-1	Black gram	Kotabagh, Nainital	23/12/20	29.28, 79.34	<i>Megalurothrips usitatus</i>	PX951424
7	LR-1	Litchi	Ramnagar, Nainital	23/03/21	29.39, 79.12	<i>Thrips coloratus</i>	PP980485
8	RD-5	Radish	Gularbhoj, Udham Singh Nagar	19/03/21	29.09, 79.31	<i>Thrips flavus</i>	PP980488
9	BB	Broad bean	Dineshpur, Udham Singh Nagar	19/03/21	29.04, 79.32	<i>Thrips flavus</i>	PQ145546
10	FN-3	Fennel	Pantnagar, Udham Singh Nagar	06/05/22	29.02, 79.48	<i>Thrips flavus</i>	PQ144647
11	MUS	Mustard	Chorgalia, Nainital	28/09/21	29.28, 79.34	<i>Thrips flavus</i>	PQ144658
12	AMR	Amaranthus	Gaulapar, Nainital	15/09/21	29.17, 79.59	<i>Thrips hawaiiensis</i>	PP971699
13	FN-1	Fennel	Kunwarpur, Nainital	15/09/21	29.21, 79.51	<i>Thrips palmi</i>	PQ144648
14	BRJ	Brinjal	Malla Pachonia, Nainital	28/09/21	29.13, 79.70	<i>Thrips palmi</i>	PQ144667

Sl. No.	Sample Code	Host Plant	Place of Collection	Date of Collection	Lat-Lon	Species of Thrips	GenBank Accession No.
15	OV-3	Onion	Chanfi, Nainital	07/09/21	29.33, 79.58	<i>Thrips tabaci</i>	PP972322

The genera *Haplothrips* and *Megalurothrips* formed distinct clades that were evolutionarily separate from the genus *Thrips*, supporting their taxonomic distinctiveness. Within the *Haplothrips* group, *Haplothrips ganglbaueri* (MZ) and *Haplothrips andresi* (KL 1) clustered closely with strong bootstrap support, indicating a close evolutionary relationship. This is consistent with the placement of both species in the subgenus *Haplothrips* (Mound and Minaei 2007; Saran et al. 2025).

Within the genus *Thrips*, several notable relationships were observed. Populations of *Thrips flavus* exhibited partial clustering patterns. Isolates FN 3 and MUS grouped closely together, indicating considerable genetic similarity, whereas RD 5 and BB occupied relatively distinct positions within the broader *Thrips* lineage. This pattern suggests moderate intraspecific genetic variation, potentially influenced by host association or geographic factors (Tyagi et al. 2017; Sharma et al. 2024).

The analysis further revealed close evolutionary affinities between certain species pairs. *Thrips palmi* (FN 1) and *T. hawaiiensis* (AMR) formed a strongly supported clade, suggesting a relatively close evolutionary relationship. Similarly, *T. tabaci* (OV 3) and *T. coloratus* (LR 1) clustered together with strong bootstrap support, indicating a shared genetic affinity. *T. tabaci* is known to be a cryptic species complex comprising several lineages (L1, L2, T) that differ in reproductive mode but are morphologically indistinguishable (Brunner et al. 2004; Farkas et al. 2019). The onion isolate (OV 3) in this study belongs to the leek-associated thelytokous (L2) lineage based on its COI haplotype (Farkas et al. 2019).

The *Megalurothrips usitatus* populations collected from cauliflower, black gram, cowpea, and fenugreek formed a closely associated cluster with limited internal divergence, indicating a high degree of genetic similarity among populations from different host plants. This clustering pattern suggests genetic homogeneity within the species, possibly due to gene flow, recent dispersal, or conserved mitochondrial sequences (Tyagi et al. 2017).

The present morpho-molecular characterisation successfully documents the diversity of thrips associated with economically important crops in Uttarakhand. The observed clustering patterns largely align with earlier molecular phylogenetic studies of Thysanoptera. The clear grouping of *Megalurothrips* and *Haplothrips* taxa supports previous findings regarding the phylogenetic distinctness of major thrips lineages (Buckman et al. 2013; Tyagi et al. 2017). Similarly, the moderate genetic variation observed among *T. flavus* populations concurs with earlier reports of host-associated variability and population structuring within thrips species complexes.

The high genetic similarity among *M. usitatus* populations from different host plants indicates a potential for broad host adaptability and dispersal capability. In contrast, the observed variation among *T. flavus*

isolates may suggest restricted gene flow or adaptation to distinct ecological niches, which could have implications for pest surveillance and management strategies.

Conclusion

This study provides a comprehensive morpho-molecular assessment of thrips diversity associated with important agro-ecosystems in Uttarakhand. A total of eight thrips species were identified and characterised using both morphological and molecular approaches, and their DNA sequences were deposited in GenBank, thereby contributing valuable genetic resources for future taxonomic and phylogenetic studies.

The phylogenetic analysis revealed distinct clustering at both genus and species levels among the studied taxa, alongside moderate genetic variation within certain species, such as *Thrips flavus*. Closely related groupings were observed among *Megalurothrips usitatus* populations collected from different host plants, indicating high genetic similarity and potential broad host adaptability. Strong evolutionary affinities were also noted between *T. palmi* and *T. hawaiiensis*, as well as between *T. tabaci* (L2 lineage) and *T. coloratus*.

The findings demonstrate the value of integrating morphological identification with molecular characterisation for accurate species delimitation and a better understanding of evolutionary relationships in thrips. Such an integrated taxonomic approach is essential for reliable biodiversity assessment and will support the development of effective monitoring and pest management strategies for economically important thrips species.

Declarations

Competing Interests:

The authors declare that they have no relevant financial or non-financial interests to disclose.

Ethics Approval:

This study did not involve human participants, animals, or any material that requires ethical approval. The research was conducted on agricultural insect pests using standard collection and identification methods, which do not require institutional ethics committee approval.

Consent to Participate:

Not applicable.

Consent to Publish:

Not applicable.

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Author Contribution

Saty Saran, Ravi Mohan Srivastava, and Srikanth N. jointly conceptualised the study and contributed to the drafting of the original manuscript. Ravi Mohan Srivastava supervised the research work. All authors critically reviewed and approved the final version of the manuscript.

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Data Availability:

The mitochondrial COI gene sequences generated during this study have been deposited in GenBank under accession numbers PQ144650, PQ144669, PP972323, PX951426, PP980482, PX951424, PP980485, PP980488, PQ145546, PQ144647, PQ144658, PP971699, PQ144648, PQ144667, and PP972322. All sequences are publicly available. Other data generated or analyzed during this study are included in this published article and its supplementary information files.

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Figures

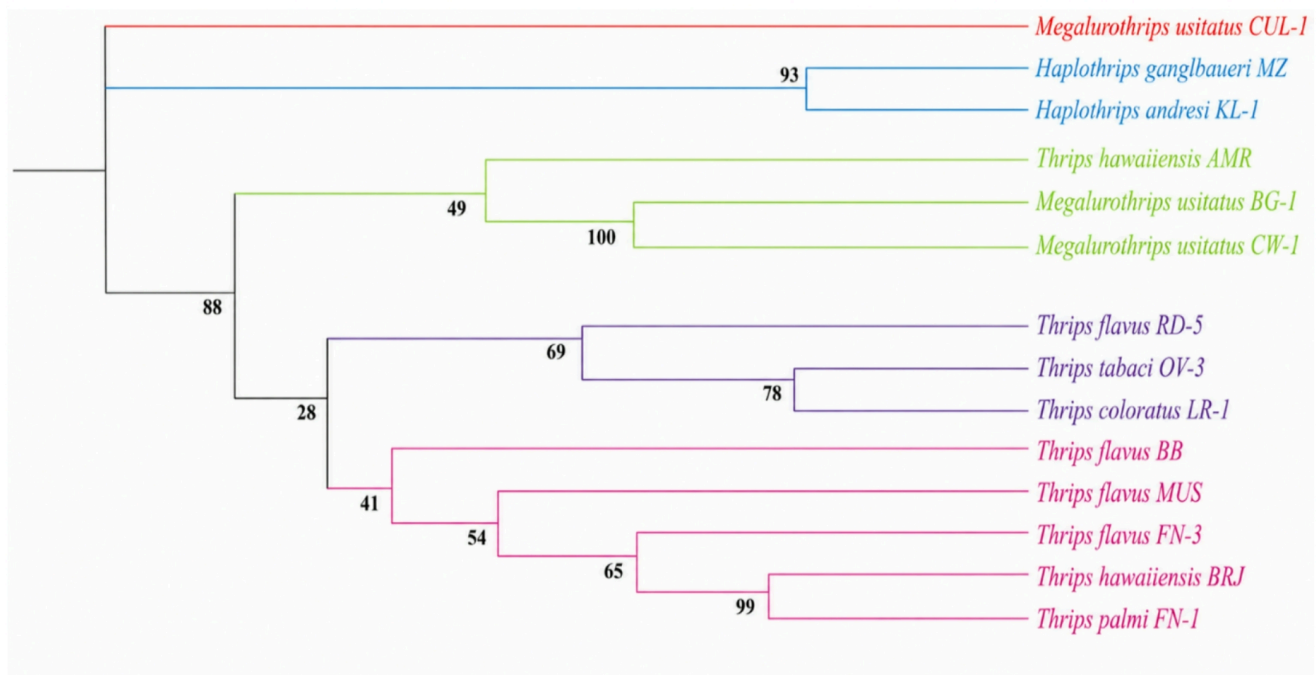


Figure 1

Phylogenetic tree (neighbour-joining, K2P) based on mtCOI sequences of thrips species from Uttarakhand. Bootstrap values ($\geq 70\%$) shown at nodes.