




DNA barcoding of selected *Scirtothrips* species (Thysanoptera) from India

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ABSTRACT

The members of the genus *Scirtothrips* are highly polyphagous, including major pest and vector species. We applied both morphology and molecular approaches to delimit the selected *Scirtothrips* species from India. Out of 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 distinguishes all the studied species with reciprocal monophyletic criteria and represents 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 indicating possible existence of cryptic diversity. The current study also provided the morphological keys for six *Scirtothrips* species including *S. hitam* as a new record to India.

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1. Introduction

Thysanoptera (commonly known as thrips) are fringe winged insect generally 0.2–15 mm in size. Globally, 6154 species were described into two suborders followed by nine families and five subfamilies (ThripsWiki 2019). India shares only 12% of the global thrips diversity, which is represented by 739 species (Tyagi and Kumar 2016). Thrips hampers the economy by damaging agricultural and horticultural crops through direct feeding or by transmitting plant pathogenic tospoviruses (family Bunyaviridae). Currently, 180 species have been reported as major sucking pests (Mound and Palmer 1981), among them 15 species are reported as a vector for tospoviruses (Riley et al. 2011; Zhou and Tzanetakis 2013). As compared with the global distribution, 54 pest species and six vector species are distributed in India (Tyagi and Kumar 2017). Thus, concerning their effect on agriculture, accurate species identification is essential for the implementation of effective integrated pest management strategies.

The genus *Scirtothrips* is one of the largest genera under subfamily Thripinae of family Thripidae. The genus encompasses 103 species from tropical and subtropical regions of the world (ThripsWiki 2019); of which, nine species are reported from India (Tyagi and Kumar 2016; Tyagi, Chakraborty, et al. 2017). The members of this genus are usually pale yellow, tiny, quick jumpers, and not easily noticeable in the wild. Among the extant species diversity, most of them are highly polyphagous while few are reported to be mono- or steno-phagous (*Scirtothrips perseae* and *Scirtothrips frondis*). Species under this genus are recorded as major pest

worldwide like, *Scirtothrips aurantii* on citrus in South Africa and on bananas in Yemen; *Scirtothrips bispinosus* on tea in India; *Scirtothrips citri* on citrus in California; *Scirtothrips dorsalis* on ground-nuts in India, on tea in Japan, and on strawberries in Queensland; *Scirtothrips inermis* on citrus in New Zealand; *Scirtothrips kenyensis* on tea in India and Kenya; *Scirtothrips longipennis* on glasshouses; *Scirtothrips mangiferae* on mango in India, Egypt, and Israel; *Scirtothrips manihoti* on cassava in Brazil; *Scirtothrips oligochaetus* on cotton in India (Mound and Palmer 1981). In last decade, the *Scirtothrips* species were causes billion of damages in both Indian agriculture and horticulture crops (Sithanantham et al. 2007; Samota et al. 2017; Bana et al. 2018; Rathee and Dalal 2018). Nevertheless, due to their pestiferous nature and high invasion potentiality, the *Scirtothrips* species are becoming a severe quarantine concern in several countries including India (Mound and Palmer 1981; Nakahara 1997; Morse and Hoddle 2006). So, for framing the suitable pest management policies and quarantine measures, accurate species identification is pivotal.

The integrated approach combining with morphology and molecular data successfully evidenced to identify the thrips species in recent past (Iftikhar et al. 2016, Tyagi, Kumar, et al. 2017). Further, this molecular tool was also effectively detected the cryptic diversity in *S. aurantii* (Rafter et al. 2013), *S. dorsalis* (Dickey et al. 2015; Rebijith et al. 2014), and *S. oligochaetus* (Tyagi, Kumar, et al. 2017) in different regions of the world. However, the scientific communities are still murmuring to identify the *Scirtothrips* species due to their minute

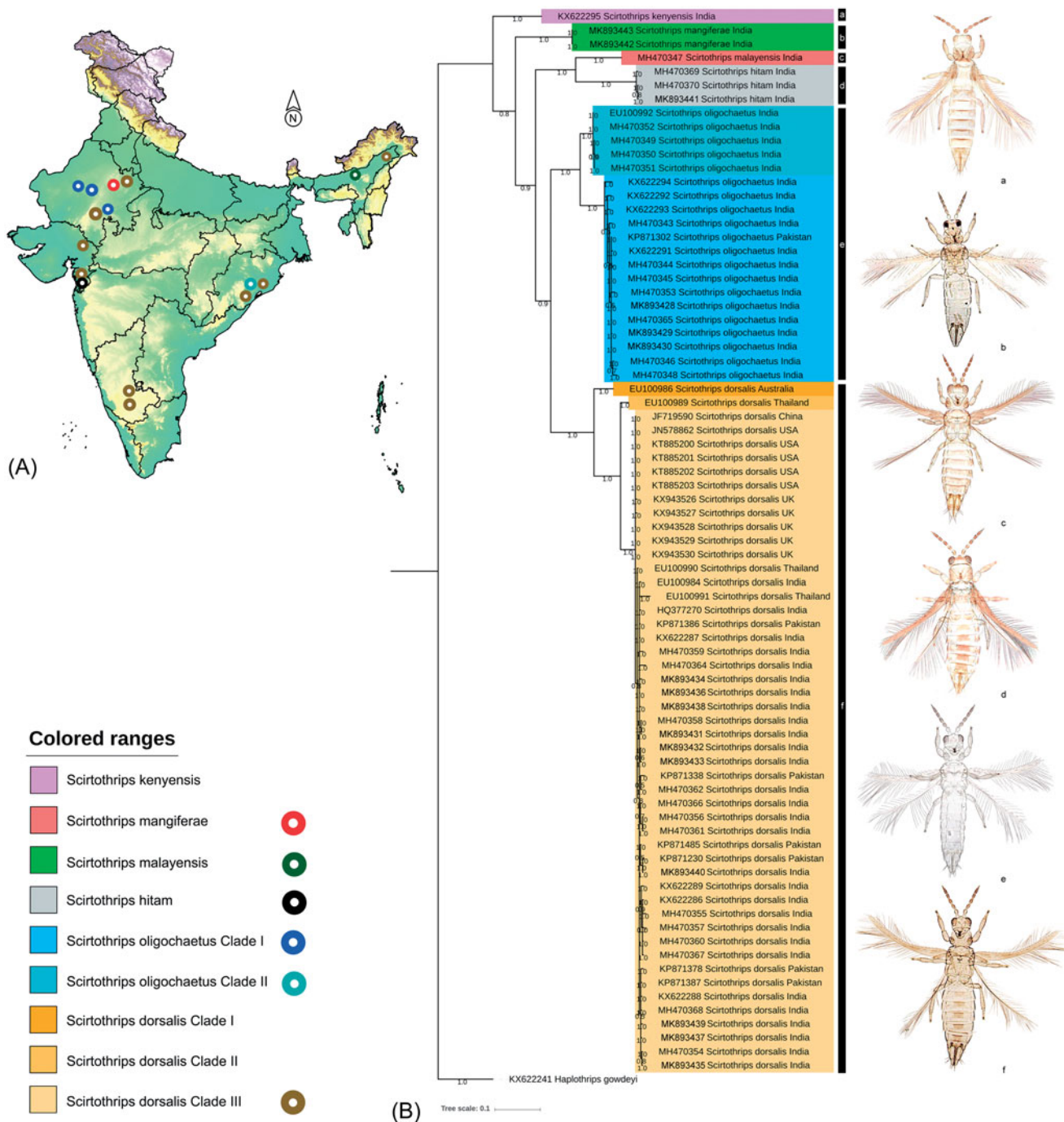


Figure 1. (A) Map showing the collection localities of *Scirtothrips* species at different states in India. (B) Bayesian phylogeny showed the monophyletic clustering of the studied species with possible cryptic diversity in *S. dorsalis* and *S. oligochaetus*. The posterior probability values are displayed with each node.

size, complex chaetotaxy, and cryptic behaviour. Besides, the DNA barcode sequences of the Indian species are limited in global database. Hence, the present study aimed to discuss the genetic identity of five *Scirtothrips* morphospecies from India with their precise keys, and DNA barcode data.

2. Materials and methods

2.1. Taxon sampling, vouchering, and non-destructive molecular interrogations

Total 43 samples were collected from the different geographic locations of India by well-known beating method

(Figure 1(A)). The specimens were preserved in 70% molecular grade alcohol for both morphological and molecular examination. No specific permissions were required as the studied species are not listed in the IUCN red data list. The specimens were made an intersegmental abdominal cut and lysed overnight in buffer ATL with proteinase-K at 56 °C with 350 rpm (revolutions per minute) in eppendorf ThermoMixer (Eppendorf AG, Germany). After tissue lysis, each specimen were retrieved and mounted onto glass slides for morphological investigation and supernatant was processed for DNA extraction. The identification was carried out by published morphological keys (Ng and Mound 2015; Ng et al. 2014).

The slide mounted specimens were photographed through the Leica software application suite (LAS EZ 2.1.0) on a Leica Trinocular Microscope (Leica DM-1000). The voucher specimens were properly labelled and deposited in the National Zoological Collections of Zoological Survey of India, Kolkata. Subsequently, the total genomic DNA was extracted by using the NucleoSpin tissue XS (Macherey-Nagel, Germany). Polymerase Chain Reaction (PCR) was performed for the amplification of partial mitochondrial Cytochrome Oxidase Subunit I (COI) gene (~648 bp) by published primer pairs: HCO-2198: 5'-TAACTTCAGGGTGACCAAAAATCA-3' and LCO-1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and thermal profile (Folmer et al. 1994). The Sanger sequencing protocol was followed as published in the previous study (Tyagi, Kumar, et al. 2017).

2.2. Sequence annotation, and dataset preparation

The generated forward and reverse chromatogram files for each specimen were checked in MEGA6 (Tamura et al. 2013), and the ambiguous bases were trimmed at both ends to make the consensus sequences. The generated sequences were further screened in BLASTn (Basic Local Alignment Search Tool) (<https://blast.ncbi.nlm.nih.gov>) and ORF finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) for the manifestation of indels (insertion/deletions) and stop codons, available in NCBI (National Centre for Biological Information) online server. The generated sequences were similarity searched in online identification engines of both NCBI and BOLD (Barcode of Life Data System) with highest percentage of identity with the publicly available sequences. To prepare a final dataset for the present analysis, 34 database sequences of three species (*S. dorsalis*, *Scirtothrips keneyensis*, and *S. oligochaetus*) were acquired from NCBI database. The dataset with 77 sequences were aligned using ClustalX software (Thompson et al. 1997), and made an equal length of 648 bp.

2.3. Genetic divergence, and phylogenetic tree interpretation

The genetic divergence was calculated using the Kimura-2-Parameter (K2P) model in MEGA6 (Tamura et al. 2013). Partition finder version 1.1.1 was used for finding the best fit model for the studied dataset. The best candidate model was found to be GTR+I+G (NST = 6) for all three codon positions with the lowest Bayesian Information Criterion (BIC) value: 29992.6900. To test the reciprocal monophyletic criteria for species delimitation, Bayesian analysis (BA) was implemented. BA was performed by using MrBayes 3.1 (Ronquist and Huelsenbeck 2003), with the Markov Chain Monte Carlo (MCMC), run for 50,000,000 generations with trees sampled every 100 generations (the first 1000 trees were discarded as 'burn in'). The MCMC analysis was stable when maximum standard deviation of split frequencies reached below 0.01 and potential scale reduction factor (PSRF) approached 1.0. The database sequence of *Haplothrips gowdeyi* (KX622235) under sub-order Tubulifera was used as an out-group in the phylogenetic study.

3. Results and discussion

3.1. Morphological identification

The *Scirtothrips* species were identified by the length of ocellar III in relation to the ocellar triangle in head; number of setae at the second vein of fore wing; distance between S1 setae on abdominal tergites, presence and absence of microtrichia on the sternites and tergites, complete or interrupted antecostal ridge on the tergites. Based on these morphological characters, the all the collected specimens were identified into five species (*Scirtothrips dorsalis*, *S. oligochaetus*, *S. malayensis*, *S. mangiferae*, and *S. hitam*). *Scirtothrips hitam* is a recently described species from Malaysia, and new to India. Further, due to the unavailability of morphological keys for *S. keneyensis*, we also include this species for combined species-level keys for total six species mentioned below:

Key to Indian six species of *Scirtothrips*

1. Abdominal tergites with pale antecostal ridge 2
- Abdominal tergites with dark antecostal ridge 3
2. Ocellar setae III situated in the line of anterior margin of hind ocelli. Male with drepane on tergite IX *mangiferae*
- Ocellar setae III situated between hind ocelli. Male without drepane on tergite IX *oligochaetus*
3. Abdominal sternites II–VI with rows of microtrichia extending fully across or at least the posterior half of the sternites; tergites II–VIII with S1 setae close together than their length 4
- Abdominal sternites II–VI with rows of microtrichia not extending medially but limited to lateral areas; tergites II–VIII with S1 setae further apart than their length 5
4. Antecostal ridge on tergites complete *dorsalis*
- Antecostal ridge on tergites interrupted medially *hitam*
5. Fore wing second vein with only one seta *malayensis*
- Fore wing second vein with three setae *keneyensis*

3.2. Molecular identification

The DNA barcoding supplemented with morphological studies as a contemporary tool for accurate species identification (Hebert et al. 2003; Iftikhar et al. 2016), detection of cryptic species (Hebert et al. 2004; Tyagi, Kumar, et al. 2017), and detect the route of invasion of the alien species (Tyagi et al. 2015; Singha et al. 2019). The generated DNA barcodes can be accessed by the NCBI with accession numbers (MK893428-MK893443; MH470343-MH470362; MH470364-MH470370). The similarity search results in the global databases (NCBI and BOLD) showed a 99–100% identical match with the same species except three species (*S. mangiferae*, *S. malayensis*, and *S. hitam*). Hence, the present study contributed six novel sequences of three *Scirtothrips* species from India. The estimated BA phylogeny depicted cohesive clustering of the generated sequences with the representative database sequences (Figure 1(B)). The phylogenetic tree displayed nine distinct lineages of six morphospecies, with multiple clades within *S. dorsalis* (Clade I, Clade II, and Clade III) and *S. oligochaetus* (Clade I and Clade II). The overall mean genetic

Table 1. The species level and Clades level K2P genetic divergence of the studied *Scirtothrips* species.

Species	Species level				Clade level	
	Mean inter-specific (%)		Intra-specific (%)		No. of estimated sub-clades	Inter-sub-clade (%)
<i>Scirtothrips dorsalis</i>				0–9.4	<i>S. dorsalis</i> Clade I	
<i>Scirtothrips oligochaetus</i>	11.5			0–8.9	<i>S. dorsalis</i> Clade II	7.7
<i>Scirtothrips kenyensis</i>	19.0	10.9		n/c	<i>S. dorsalis</i> Clade III	8.8
<i>Scirtothrips hitam</i>	15.1	17.2	18.8	0–1.0	<i>S. oligochaetus</i> Clade I	
<i>Scirtothrips mangiferae</i>	17.6	14.4	15.2	19.9	<i>S. oligochaetus</i> Clade II	6.4
<i>Scirtothrips malayensis</i>	17.2	14.1	14.0	16.5		18.3
				n/c		

n/c: not able to calculate due to single sequence.

divergence was estimated to be 7.8% in the present dataset. The intraspecific genetic divergence ranges from 0% (*S. mangiferae*) to 9.4% (*S. dorsalis*). However, the highest inter-specific genetic divergence (19.9%) was observed between *S. mangiferae* and *S. hitam*, and lowest inter-specific genetic divergence (10.9%) between *S. oligochaetus* and *S. kenyensis* (Table 1). The three clades of *S. dorsalis* depicted 4.5–8.8% genetic divergence, and two clades of *S. oligochaetus* showed 6.4% genetic divergence in the present dataset (Table 1). Previous studies also described the presence of cryptic diversity within *S. dorsalis* and *S. oligochaetus* (Rebijith et al. 2014; Tyagi, Kumar, et al. 2017) and suggested to deal with more specimens to validate this concealed diversity. Hence, the present study with more samples of these two species and distinctive clustering in BA phylogeny with high genetic divergence confirmed the presence of cryptic diversity in both *S. dorsalis* and *S. oligochaetus*. Further, the specimens of *S. oligochaetus* collected from two different geographical locations revealed two distinct clades suggesting allopatric speciation within India. Altogether, this preliminary study successfully identified the selected species of the genus *Scirtothrips* from India by both classical and molecular taxonomy. These humble contributions of barcode data in global database also represent three major pest species and one vector species of thrips. However, in-depth taxonomic studies of more species need to be intended with multiple molecular markers for elucidating the thrips systematics research. This similar approach could be adopted for identifying other pest and vector species and help to implement the biological, chemical and cultural control plans to protect the agriculture and horticulture crops.

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No potential conflict of interest was reported by the authors.

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