SHORT COMMUNICATION

Morphological and DNA Barcoding Evidence for Invasive Pest Thrips, *Thrips parvispinus* (Thripidae: Thysanoptera), Newly Recorded From India

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ABSTRACT. South East Asia pest thrips species, *Thrips parvispinus* (Karny), is a serious pest on a number of agricultural and horticultural crops in a number of plant families. Based on an integrated approach of morphology and DNA barcoding, invasion of this serious pest is reported first time from India on papaya plantations. Molecular data have corroborated with the morphological identification. Haplotyping data suggested that the Indonesia may be a probable source of invasion of this pest to India.

Key Words: invasive pest, thrips, COI, new record, papaya

Members of insect order Thysanoptera with two recognized suborders, the Terebrantia and Tubulifera, are commonly called thrips. Out of nearly 6,000 known species, a few have been documented as economically important, i.e., pollinator, predator, pest, and vector for plant viruses (Lewis 1997, Pappu et al. 2009, ThripsWiki 2015). As insect vectors, thrips are sole transmitters of Tospoviruses (genus *Tospovirus*, family Bunyaviridae) affecting a number of plant species in unrelated plant families across the globe (Riley et al. 2011). The peculiarity in tospoviruses transmission by thrips is that larvae can acquire the viruses, while adult can transmit (Whitfield et al. 2005).

Hence identification of both adults and larvae thrips is of prime importance for pest and vector management. Considering their minute size, cryptic behavior, high degree of similarity of various developmental stages, polymorphism, intraspecific variations, and complex morphology make morphological characters of limited use to nonspecialist for accurate and speedy identification especially those of pest and vector species (Asokan et al. 2007). Nuclear markers like ribosomal ITS have also been used for molecular identification in thrips (Toda and Komazaki 2002, Rugman-Jones et al. 2006, Farris et al. 2010). However, mitochondrial COI (DNA barcoding, Hebert et al. 2003) has been found to be the most suitable for molecular identification within the genus *Thrips* (Glover et al. 2010). DNA barcoding has been widely used in thrips identification (Rugman-Jones et al. 2010, Kadirvel et al. 2013), development of species specific markers, and phylogenetic analysis (Buckman et al. 2013). Integration of both morphological and DNA barcoding data may be immense use for accurate species identification and phylogenetic analysis (Mound et al. 2010).

Thrips parvispinus, a member of "*Thrips orientalis* group" (Mound 2005), is a widespread pest thrips species of quarantine importance and has been recorded from Thailand to Australia (Mound and Collins 2000) as a serious pest on a number of unrelated plant families. It is reported on papaya in Hawaii, greenhouse *Gardenia* plants in Greece, vegetable crops like chili, green beans, potato, and eggplant from other countries (Murai et al. 2009). Identification of an invasive pest is the first step which unlocks the barriers for further research in planning appropriate management strategies for the pest involved. The objective of this article is to report invasive pest, *T. parvispinus* from India based on specimens collected on papaya and elucidation of probable source of invasion.

Materials and Methods

Specimens were collected and stored in 70% ethanol at −80°C. DNA isolation and amplification of partial fragment of mtCOI gene, purification of amplified PCR products, and sequencing were performed as earlier protocol using non-destructive techniques (Buckman et al. 2013, Kumar et al., 2014). Voucher specimens have been retrieved after DNA isolation and mounted in Canada balsam onto glass slides and identified as *T. parvispinus* using the morphological keys (Mound 2005). The voucher specimens have been submitted to National Zoological Collections, Zoological Survey of India, Kolkata, India.

Eleven DNA sequences generated in this study were aligned against 80 sequences of *T. parvispinus* from Indonesia as retrieved from National Centre for Biotechnology Information. Further, the generated sequences were submitted to GenBank database to acquire the accession numbers for *T. parvispinus* (KM485659–KM485667) and *Thrips orientalis* (KM507077–KM507078). *T. orientalis* is used as it is a member of the "*Thrips orientalis* group." *Frankliniella schultzei* (KC513151) is used as outgroup. Evolutionary genetic divergences with Kimura-2-parameter model and neighbor-joining (NJ) phylogenetic tree were constructed in MEGA 6.0 with 1,000 bootstrap replications (Tamura et al. 2013). Haplotyping was carried out in DNaSP, and median joining networks were produced in Network 4.1 (Bandelt et al. 1999, Librado and Rozas 2009).

Results and Discussion

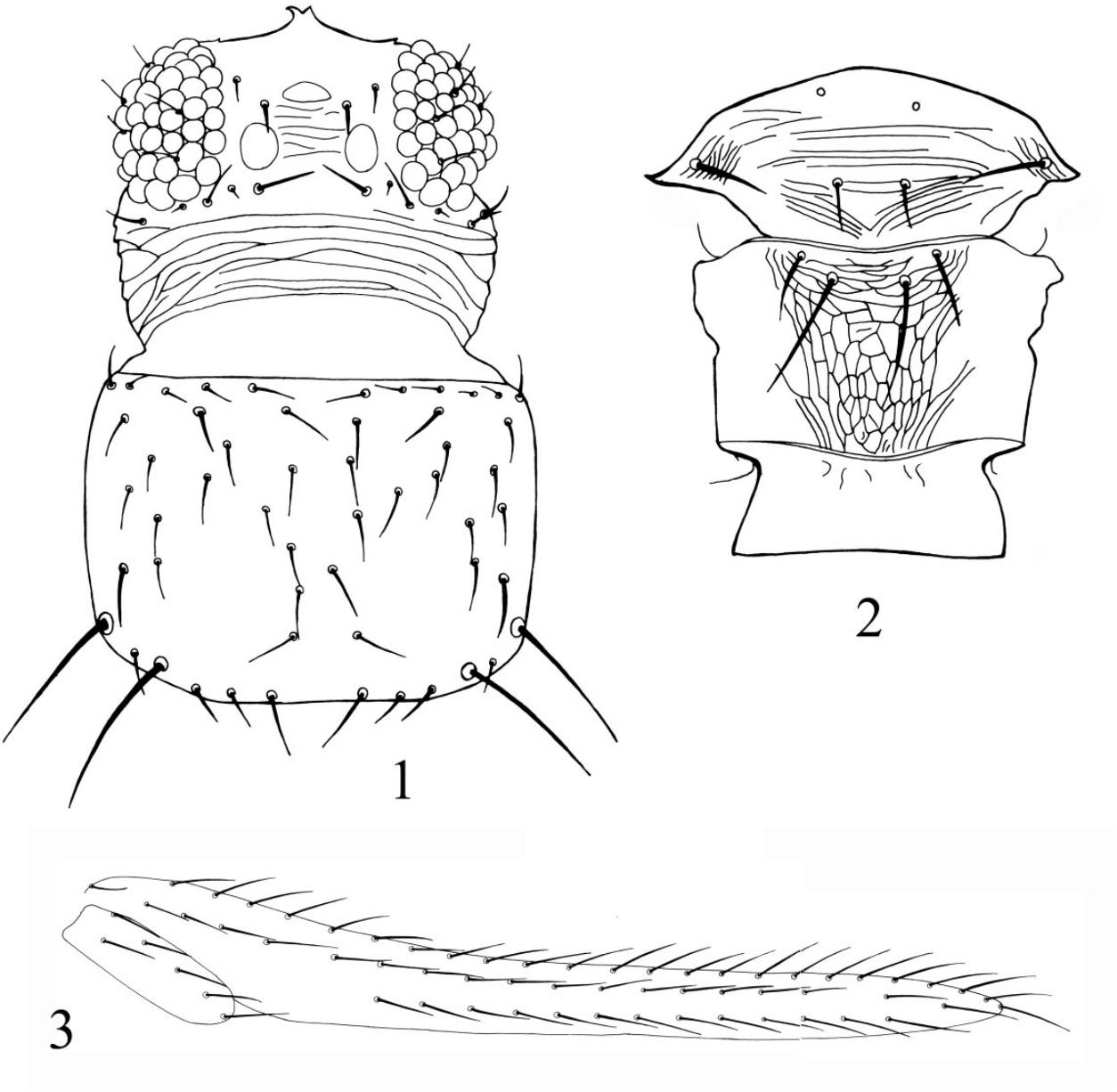
Morphological Data. *Thrips parvispinus* (Karny)

Isoneurothrips parvispinus Karny, 1922: 106.

Diagnosis. Body brown, head, and thorax paler than abdomen. Legs yellow, forewings brown with pale base. Head broader than long, ocellar pair III arising at the anterior margin of ocellar triangle; postocular setae I and IV longer than III (Fig. 1). Antennae seven segmented, segments III and IV each with forked sense cone. Pronotum with two pairs of posteroangulars setae and two pairs of posteromarginal setae (Fig. 1). Metanotum reticulate medially and with faint internal reticules; median setae long and placed behind the anterior margin; campaniform sensilla absent (Fig. 2). Fore wing first and second vein with continuous row of setae (Fig. 3). Posterior margin of tergite VIII without comb, a few microtrichia present laterally. Abdominal sternites III–VI with accessory setae, absent on II and VII.

Male. Body yellow. Posterior margin of tergite VIII without comb. Abdominal sternites II–VI each with pore area.

Material examined. Thirteen females, two males, India: Karnataka: Bangalore, 10.ii.2014, Kamlajayanti (Reg. No. 5618/H17 to 5628/H17, 5994/H17, 5998/H17, 5999/H17, 6000/H17 to 6001/H17).



Figs. 1–3. *T. parvispinus*, female (1) head and pronotum; (2) meso- and metanotum, (3) fore wing.

Molecular Data

Homology search using BLAST search option resulted in 99–100% similarity to *T. parvispinus* sequences from Indonesia. We analyzed 91 partial mtCOI sequences of *T. parvispinus* and *T. orientalis* in this study. Out of which 11 sequences were generated in this study and rest of the 80 sequences were retrieved from National Centre for Biotechnology. Complete dataset after trimming have 604 nucleotides, which shows 89 variable sites with 87 parsimony informative sites. However, the dataset of 89 sequence of *T. parvispinus* yielded only 14 variable sites out of which 10 were parsimony informative.

Analysis of 89 sequences of *T. parvispinus* yielded four haplotypes (Hap_1 to Hap_4) (Fig. 4). Hap_1 and Hap_3 were from Indonesia. Hap_2 includes 18 sequences from India and Indonesia. Hap_4 is represented by a single specimen from India (Table 1). The data show that there is no host-plant or geographical locality specific haplotyping. The total number of segregating sites between four derived haplotypes varies from 1 to 13 (Table 1). Further, out of 14 segregating sites, 11 were detected as synonymous changes and 3 as nonsynonymous changes corresponding to nine transitions and five transversions. The analysis of NJ tree yielded two major clades with high bootstrap support; clade I includes 89 sequences of *T. parvispinus* from Indonesia and India, while clade II is represented by two sequences of *T. orientalis* (Fig. 5). *F. schultzei* is used as outgroup. NJ tree provided here is only to segregate two species based on the reciprocal monophyly criteria and not to interpret phylogeny of genus *Thrips*.

Both morphological and molecular evidences verify that the specimens collected on papaya represents *T. parvispinus*. The presence of this pest species on an economically important crop plant like papaya in India raise serious issues and is a concern for quarantine authorities. Occurrence of *T. parvispinus* in other parts of India needs systematic monitoring as it is likely to acquire pest status in future. Molecular evidence for shared haplotype (H_2) between Indonesia and India indicated that there is a flow of genetic material, and Indonesia may be a probable source of invasion of this species to India. However,

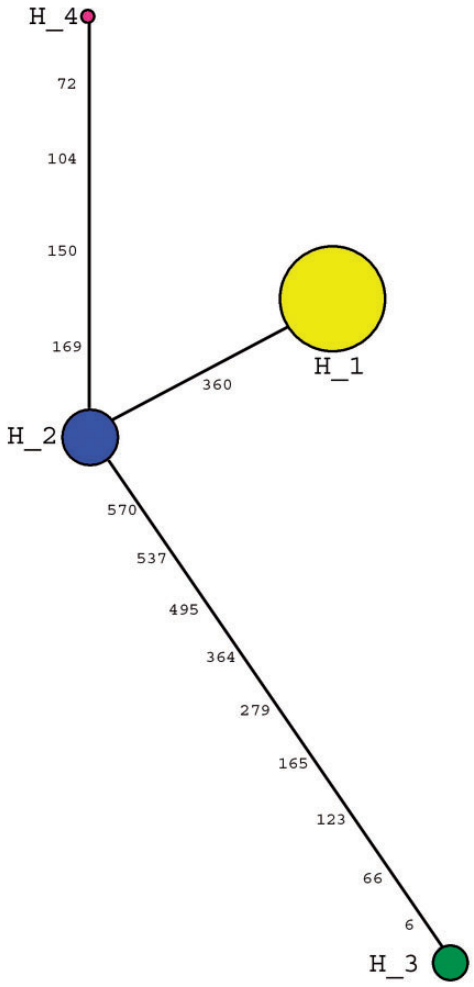


Fig. 4. Median joining networks shows four different haplotypes of *T. parvispinus* by COI sequences.

Table 1. Segregating nucleotide sites in the *T. parvispinus* mtCOI nucleotide sites as depicted in four haplotypes

Haplotypes	16	66	72	104	123	150	165	179	279	360	364	495	537	570
Hap_1 (Indonesia), *n* = 63	C	A	**T**	A	T	**T**	A	**G**	A	T	T	T	**A**	**T**
Hap_2 (Indonesia, India), *n* = 18	.	.	.	.	.	.	.	.	.	C	.	.	.	.
Hap_3 (Indonesia), *n* = 7	T	G	.	.	C	.	G	.	G	C	C	C	T	A
Hap_4 (India), *n* = 1	.	.	G	G	.	G	.	T	.	C	.	.	.	.

Transversions are shown in bold. A dot indicates an identical nucleotide with respect to Hap_1.

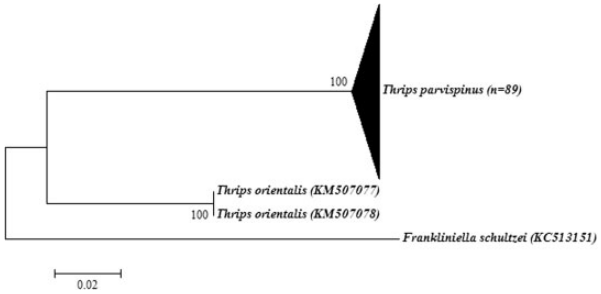


Fig. 5. NJ tree of *T. parvispinus* and *T. orientalis*. *F. schultzei* is used as an outgroup.

molecular data on this species from other countries may be helpful to trace exact route of invasion.

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