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Morphological and Molecular Confirmation of *Thrips palmi*, Karny 1925 (Thripidae) and *Haplothrips tenuipennis* Bagnall, 1918 (Phlaeothripidae) of Order Thysanoptera in Muthalamada Mangoes of Kerala, India

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***Abstract*:** Periodic incidence of sucking pests in Muthalamada mangoes, Palakkad, Kerala has caused severe setback to mango growers. Symptoms of damage include malformation of mango panicles and bronzing of mango fruits which resulted in the reduced marketability of the produce. A random survey was conducted in mango orchards of these regions during the flushing, flowering, and fruit setting stage of crop during September 2022 to May 2023. Collected thrips specimens were subjected to morphological identification done with permanent slide technique of taxonomic key characteristics. Because *T. palmi* Karny and *Haplothrips tenuipennis* Bagnall has close physical resemblance to other thrips species, we used the mitochondrial cytochrome oxidase 1 (mtCO1) gene as a molecular marker. BLAST analysis of this sequence allowed us to identify the collected specimen as *T. palmi* and *H. tenuipennis*.

Keywords: Muthalamada, Mango city, Mitochondrial COI, *Thrips palmi, Haplothrips tenuipennis*

Palakkad district of Kerala is the mango hub ('mango city') with have maximum production of 55120 MT and area of 10068 ha (Ecostatkerala 2020). Kerala is also a hotspot for climate change and variability particularly Muthalamada experiences a lot of climatic variabilities which causes fluctuations in the population of insect pests mainly thrips. Thrips causes scars on the surface of fruit and foliage in addition to feeding and reproduction on inflorescences (Kumar et al 2012). *T. palmi* Karny and *H. tenuipennis* Bagnall was reported on mango inflorescence from India (Tandon and Verghese 1987 and Ramasubbarao and Thammiraju 1994). Krishnamoorthy and Visalakshi (2012) reported that of the several sucking pests infesting mango inflorescence, *T. palmi* and *H. tenuipennis* caused considerable loss. The unique properties of thrips make morphological key identification a laborious process that requires accuracy and speed (Asokan et al 2007). Nuclear markers such as ribosomal ITS have been utilized for molecular identification (Farris et al 2010). Nevertheless, it has been found that the best method for molecular identification within the genus Thrips is mitochondrial COI (DNA barcoding). The objective of paper is to confirm the presence of *T. palmi* and *H. tenuipennis* through morphological key characters and molecular DNA barcoding (Asokan et al 2007) and NJ (neighbour joining) or maximum likelihood analyses help for phylogeny study of such species.

MATERIAL AND METHODS

Sampling and morphological identification: Random survey was conducted in five orchards (Table 1) of Muthalamada panchayat, Palakkad, Kerala during the year 2023 provided a sample, which thrips were collected from five randomly chosen trees in each orchard by adopting CO_2 method (Aliakbarpour and Che Salmah 2010). The samples were subsequently stored in 70% alcohol, and a stereomicroscope was used to sort each individual thrips. Adult thrips were mounted using procedure of permanent slide technique (Mound 2005). The specimens were divided into suborders, Terebrantia and Tubulifera and placed in vials containing 70% alcohol before being transmitted to the NBAIR and ZSI for species confirmation.

DNA Barcoding using Universal Primers of COI

Genomic DNA isolation: Using the NucleoSpin® Tissue Kit (Macherey-Nagel) and the manufacturer's instructions, genomic DNA was extracted from the tissues. The extracted DNA stored at – 20º C. PCR was carried out with universal primer forward primer (LCO1490) 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer (HCO2198) 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Vrijenhoek 1994), to amplify a 478 bp and 483 bp fragment of COI gene for *T. palmi* and *H.tenuipennis*. PCR amplification was carried out by following Thermo Scientific Phire Plant Direct PCR Master Mix product's instruction

(http://assets.fishersci.com/TFS-Assets/LSG/manuals/F-=160_QR_TS_5.PDF). Purification of amplified product checked by 1.2 % agarose gel with ethidium bromide (Buckman *et al*. 2013 and Kumar *et al*. 2014). The products that were amplified were sent to the Rajiv Gandhi Centre for Biotechnology (RGCB) in Trivandrum, Kerala, for sequencing (India).

The National Center for Biotechnology Information (NCBI) database's Basic Local Alignment Search Tool (BLAST) was used to run a similarity search to determine the identity of the genomic DNA. The sequences were then compared with an existing database to confirm the species. Furthermore, additional sequences were obtained from GenBank and a multiple alignment was done using ClustalX version 2.1 (Thompson et al 1997). In addition, extra sequences were dragged from GenBank, and ClustalX version 2.1 (Tamura et al 2021) was used to perform a multiple alignment. Using the Jukes-Cantor distance model and the Maximum Likelihood approach with a bootstrap test of 1000 replications, the phylogenetic tree was studied using MEGA version 11.0. (Jukes and Contar 1969).

RESULTS AND DISCUSSION

Morphological Confirmation

Phylum: Arthropoda

Class: Insecta

Order: Thysanoptera

Family: Thripidae

Sub family: Thripinae

Genus: *Thrips*

Species: ***T. palmi* (Karny) 1925:** The following characteristics distinguish *T. palmi* from other known species of the genus Thrips during the morphological key characterisation process, which was carried out using created permanent slides of thrips (Fig. 1a-g). Head is broader than long; Ocellar seta III faces lateral on anterior ocellus (Fig. 1g); antenna 7- segmented, I-II yellowish, III – V basally yellowish and distally brown, VI-VII brown (Fig 1b); Pronotum with 2 pair of long posteroangular setae (Fig 1d); Metanotum with two companiform sensilla (Fig 1e); Forewing yellow with three (sometimes two or four) setae on distal half of first anterior vein, Second vein with row of 15 setae approximately (Fig 1f). Abdomen tergite II with four lateral setae and microtrichia lacking on lateral thirds of tergites IV–VI; Tergite V –VII with paired ctenidia laterally; Tergite VII Posteromarginal comb complete, microtrichia long and slender (Fig 1c); Discal setae absent.

Phylum: Arthropoda

Class: Insecta

Order: Thysanoptera

Family: Phlaeothripidae

Sub Family: PhlaeothripinaGenus: *Haplothrips*

***Species*: *H. tenuipennis* Bagnall**, 1918: 210: *Haplothrips ceylonicus var. mangiferae* Priesner, 1933: 359 syn.n. All members of the genus Haplothrips have four sense cones (rarely three) on segment IV of the antenna and one or two on segment III of the antenna. In *Haplothrips tenuipennis*, S1 setae in tergite not more than 0.75mm as long as tube. Antenna; the antennal segment III has a smaller sense cone and thinner. Sub basal S3 seta of forewing is acute (Mound 2019), which were confirmed the attack of mango inflorescence by *T. palmi* and *Haplothrips tenuipennis.* Zambrano et al (2021) first reported *T. palmi* on cotton in Ecuador by using the taxonomic key characters. Krishnamoorthy and Ganga Visalakshi (2009) reported *T. palmi* on flowers in his trail field to manage mango hoppers and thrips using entomopathogens. Additionally, *H. tenuipennis* was found in the same host from Andhra Pradesh, India by Ramasubbarao and Thammiraju 1994; Kannan and Rao 2006a, b). Earlier, *S. rubrocintus* has been

Table 1. Location of orchards covered during survey 2023

State	District	Panchayat	Locality	Latitude, Longitude	Host	Specimen	Date of collection	Name of collector	Material deposited
Kerala	Palakkad	Muthalamada	Adavumaram	10.5992614 76.7621706	Mango	*T. palmi* and *H. tenuipennis* (Adult thrips)	04/12/2023	Syed Mohamed Ibrahim	Insect museum, College of agriculture, Vellayani
			Vellaramkadavu	10.5740950 76.7725330			04/12/2023		
			Vellaramkadavu	10.5772010 76.7762450			08/12/2023		
			Anna nagar	10.5792830 76.7786070			08/12/2023		
			Chemmanapathy	10.57927 76.778582			13/12/2023		

observed in Kerala on inflorescence of mango (Ananathakrishnan and Muraleedharan 1974). But, till now no other thrips species was reported on mango in Kerala, India. Verghese et al (1988) and Krishnamoorthy and Visalakshi (2009) had previously reported on *T. palmi*'s attack on mango inflorescence in Tamil Nadu. On the other hand, Kerala is the first place where reports of these two species attacking mango inflorescences have been confirmed.

Molecular Characterisation

In the study, the NCBI database was used to BLAST sequences generated over incomplete mtCOI areas in order to obtain the accession numbers (OP963194 and OP957024) for *T. palmi* and *H. tenuipennis* from Muthalamada. With an E-value of 0 and a likelihood of 92.79% and 92.89% match, the Blast search using the NCBI BLAST connected the individuals to the *T. palmi* and *H. tenuipennis* sample from China and Pakistan, accession numbers MF686687 and KP871477. The sum of branch length of optimal maximum likelihood tree = 0.015689 and 0.015939 is displayed in Figure 2 & 3. Eight nucleotide sequences for *T. palmi* and *H. tenuipennis* were analyzed. First, second, third, and noncoding codon locations were covered. Every ambiguous location was removed for every sequence pair (pairwise deletion option). The completed dataset contained 478 and 483 positions in total. Evolutionary analyses of maximum likelihood tree and *T. palmi mtCOI* gene sequence (collected from GenBank) revealed that our samples were closest to the *T. Palmi* of China, Bangladesh and Indonesia formed as separate cluster. *mtCOI* sequence of *T. major* was taken as outgroup (Fig 2). Similarly, the maximum likelihood tree analyses for *H. tenuipennis* displayed that the sample from Kerala was close near to the Bangladesh which occupied in one clade and the sample from India and Pakistan of GenBank occupied on different clade. *Haplothrips ganglbaueri* as taken as outgroup (Fig. 3).

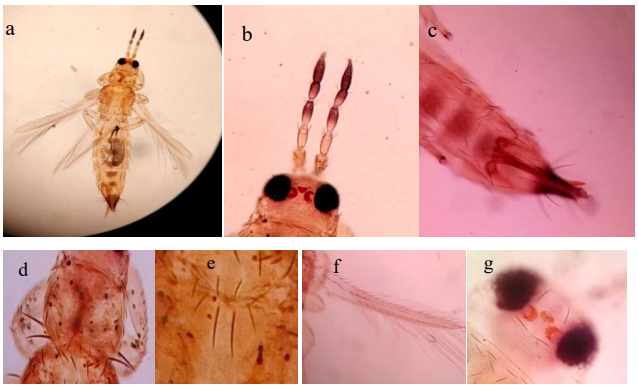

Fig. 1(a-g). *Thrips palmi*. **a**, Female; **b**, antenna; **c**, abdominal tergite; **d**, pronotum; **f**, meso and metanotum; **g**, forewing; **e**, ocelle setae

T. palmi morphologically resembles both *T. alatus* Bhatti and *T. fabus* Schrank. The location of the interocellar setae, which in *T. palmi* originate from outside the ocellar triangle but in *T. fabus,* originate from inside the ocellar triangle posterior to the anterior ocellus, supports differentiation (Mound 2009). Unlike *T. palmi,* where the metanotal sculpture is typically convergent on the posterior margin, *T. alatus* exhibits striate metanotal sculpture (Mound 2009). Likewise, female paratypes of *H. ceylonicus var. mangiferae* and syntypes of this species from northern India have been examined and compared. Mound (1968) established the first synonymy between these two species, whose females cannot be distinguished from one another. *H. tenuipennis* resembles members of the *H. anceps* group of species in general because it has dark pronotal major setae in common with them, but it differs from them in that it has a small, slender sense cone on the inner margin of antennal segment III that

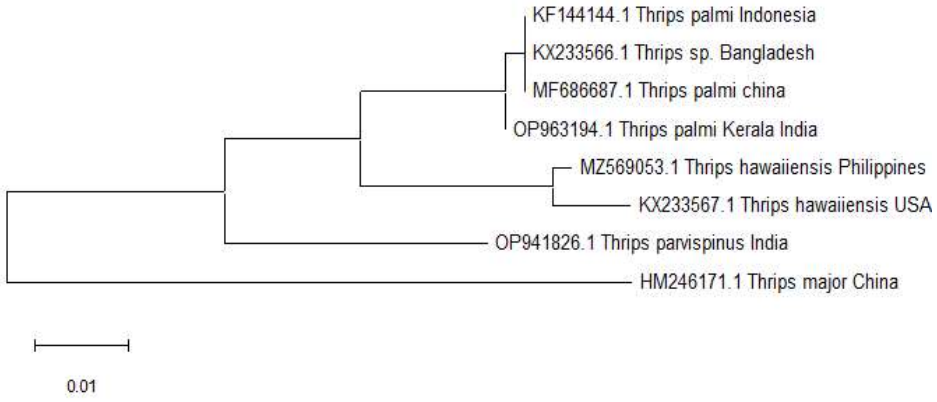

Fig. 2. Maximum likelihood tree of *T. palmi* samples from Muthalamada, Kerala, and other locations retrieved from GenBank

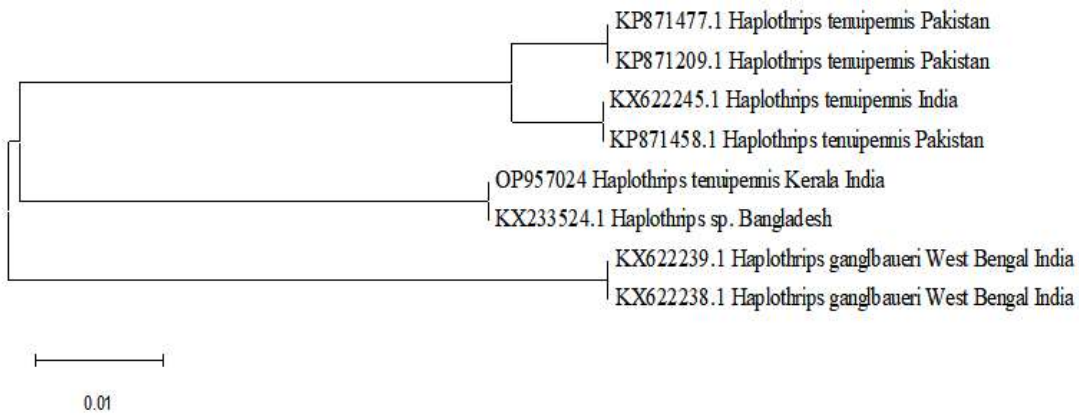

Fig. 3. Maximum likelihood tree of *H. tenuipennis* samples from Muthalamada, Kerala and others extracted from GenBank

neither of the other two species have developed. In order to prevent misunderstandings, specimens that had been recognized morphologically were next subjected to molecular identification using mtCO1 gene PCR amplification to confirm that they were as *T. palmi* and *H. tenuipennis* not any other thrips species (Riley et al 2011).

CONCLUSION

The specimens collected on mangos were confirmed to be *T. palmi* and *H. tenuipennis* by both morphological and molecular evidence. This is the first report of *T. palmi* and *H. tenuipennis* on mango inflorescence at Muthalamada panchayat in Palakad, Kerala, India. The southern states of India are ideally suited for thrips' occurrences on mango, Since *T. palmi* and *H. tenuipennis* are spreading their host range, their occurrences in various regions of India require rigorous monitoring and IPM measures.

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