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Indian record of the old world psyllid *Heterotrioza chenopodii* (Reuter) (Hemiptera, Psylloidea, Triozidae) on quinoa *Chenopodium quinoa* (Amaranthaceae)

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ABSTRACT: The Old-World Psyllid, *Heterotrioza chenopodii* (Hemiptera, Psylloidea, Triozidae) was found infesting quinoa, *Chenopodium quinoa* (Amaranthaceae) crop. The psyllids were identified using taxonomic traits and further confirmation with mitochondrial marker based molecular approach. The information on damage and its life stages is reported along with the phylogenetic information of the species. The identity analysis in NCBI indicated that the MT-COI sequences of *H. chenopodii* were 96 per cent identical to the previously deposited sequences with NCBI and five submissions with accession numbers were made *viz*., OP735496, OP740826, OP740828, OP740829 and OP740830. The phylogenetic tree represented the similarities in the analyzed sequences Indian populations are found to be merged in between the other similar global populations.

KEYWORDS: Triozid , taxonomic traits, MT-COI analysis, phylogenetic information

INTRODUCTION

Jumping plant-lice (psyllids), belong to Hemiptera; Psylloidea, are phloem sap-sucking insects that severely damage their host plants (Burckhardt *et al.,* 2006 a,b; Dzokou *et al.,* 2009). Approximately 4000 species in eight families so far around the world are described in Psylloidea and primarily habituated in the tropics and southern temperate zones (Burckhardt and Ouvrard 2012; Spodek *et al.,* 2017; Burckhardt *et al.,* 2022).The nymphs and adults inject their saliva into plant tissues, causing the entire plant to degenerate, leaves to warp, and leaves and stems to necroses (Dzokou *et al.,*2009). They make the plants sick and excretes honeydew which invites sooty mould growth, impairing plant growth (Burckhardt *et al.,* 2004). Numerous species of psyllids have the ability to cause galls in their host plants. Other psyllids are also known to be the carriers of bacterial and phytoplasma plant diseases (Mathur, 1975; Burckhardt and Lauterer, 1997).

The species from Triozidae family, *Heterotrioza chenopodii* (Reuter, 1876) reported in this study, is associated with Amaranthaceae (Halperin *et al.,*

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1982; El Nasr and Abd-Rabou, 2012). It feeds on a variety of Amaranthaceae plants including *Amaranthus* sp., *Atriplex halimus*, *A. tatarica*, *Chenopodium album*, *C. glaucum*, *C. quinoa*, *Halimione portulacoides*, *Beta vulgaris* and *Spinacia oleracea* (Mathur, 1975; Aguiar and Martin, 1999; Spodek *et al.,* 2017; Ouvrard, 2020). Initially recorded from Finland (Reuter, 1876), *H. chenopodii* is now globally reported in new geographical locations (Mathur, 1975; Horton *et al.,* 2018; Mifsud, 2020; Ouvrard, 2020; Percy *et al.,* 2020; Haouas *et al.,* 2021; Soliman *et al.,* 2021). *H. chenopodii* induces leaf deformations and yellowing on the host plants. Early-instar nymphs induce the plant tissue to produce galls on their hosts while feeding from within the leaf folds, whereas fourth and fifth instars feed freely on leaves, stems, petioles and inflorescences (Lauterer, 1982). The nymphal stage rarely moves and prefers feeding on occluded surfaces. At severe stages of infestation crop death was also observed (Soliman *et al.,* 2021). In this paper, the occurrence of *H. chenopodii* into southern India is reported. The species identity was confirmed by examination of morphological traits and molecular markers, mitochondrial cytochrome oxidase I gene (MT-COI). The sequences were deposited to National Centre for Biotechnology Information (NCBI) to obtain accession numbers.

MATERIALS AND METHODS

Specimen collection: Adult and immature stages of *H. chenopodii* were collected from *Chenopodium quinoa* plants grown in TNAU, Coimbatore (Latitude: 11.008114N, Longitude: 76.932394E) during August 2022. Psyllids were collected by tapping infected plants over a white beating sheet to dislodge different lifestages and aspirating dislodged insects into vials (Fig. 1). The collected psyllids were transferred using a fine brush to a labelled vial containing absolute ethanol. The collected specimens were stored under -20^{o}C until further processes. The collected live specimens were immobilized at 4 ^{o}C for 5 minutes and then, the photographs of live adult and immature insects were taken using Leica M-205A Encoded Stereo Microscope (Leica Microsystems, Wetzlar, Germany).

Morphological trait examination: The specimens were cleared for mounting onto microscope slides by immersion in KOH (10%) at room temperature and then washing with distilled H_2O. The terminal segments of the abdomen were removed from a specimen using insect pins and placed in lactic acid on a microscope slide. The structure was photographed at 4, 10, 40 and 100X using a phase contrast microscope (Euromex iScope, The Netherlands). Measured 10–15 specimens of both sexes. The mounted specimens were identified using descriptions as found at https://bugguide.net/node/ and the standard morphological keys down to species level given by Wheeler and Hoebeke (1997). Confirmed the identification of *H. chenopodii* from the descriptions and illustrations provided for Old-World populations of psyllid (Lauterer, 1982).

Diagnostic traits included wing venation; shape and size of the egg; adult body coloration, leg and antennal colour and morphology of the fifth instar nymph, including features of the marginal sectasetae. Measurements of the adult, egg and fifth-instar nymphs were made using the Leica M-205A Encoded Stereo Microscope equipped with an ocular micrometer. The measurements were taken largely to supplement descriptions of

H. chenopodii available in the literature. Total body length was measured for the fifth-instar nymph by placing specimens in a drop of alcohol on a microscope slide beneath a cover slip. Marginal sectasetae were examined in fifth-instar nymphs by placing a specimen dorsal side up on a microscope slide in KOH (10%). The slide was gently heated for 1 h to dissolve wax filaments. Number of marginal sectasetae on the head, forewing pad, hindwingpad, and abdomen were determined by examination of the digital images.

DNA isolation: Total genomic DNA was extracted from egg, nymph and adult psyllids separately by a rapid standard method (Montero Pau *et al.,* 2008). Briefly, a single specimen in a micro centrifuge tube was ground using 30 ìl of tissue lysis buffer (10N NaOH and 0.5M Na EDTA at pH 8.0) by crushing the sample and homogenized. The lysate was mixed by vortex and the homogenate was incubated at

65°C for 30 min. To inactivate the lysis buffer, an equal volume of neutralizing buffer (10 mM Tris-HCL, pH 5.0) was added and incubated at 95°C for 15 min followed by centrifugation for 5 min at 12000 rpm. Finally, the DNA aliquot was stored until further processes. Quantification of DNA was done in NanodropOne™ (Thermoscientific, Massachusetts, United States) at A260 nm before polymerizing reactions. The MT-COI was amplified using the primers LCO (5' GGTCAACAAATCATAAAGATATTGG 3') and HCO (5' TAAACTTCAGGG TGACCAA AAAATCA 3') in polymerase chain reaction (PCR). PCR conditions of initial denaturation at 94°C for 5 min, followed by 30 cycles of (i) denaturation at 94°C for 1min, (ii) annealing at 50°C for 1min, (iii) elongation at 72°C for 1min and a final extension step at 72°C for 10 min in Eppendorf Mastercycler™ thermocycler (Eppendorf, Hamburg, Germany) (Hoy and Jeyaprakash, 2005). The amplified DNA products were resolved on 1 per cent agarose gel stained with ethidium bromide (10 mg/ml) and visualized in a gel documentation system (Bio-Rad®, Hercules, USA) for quality and then, the obtained PCR product after purificationwas checked for quantity in NanodropOne™ at A260 nm. Then, it was sent to the sequencing facility at Eurofins India Pvt. Ltd., Bangalore, India, and was sequenced in both directions utilizing double pass sanger dideoxy DNA sequencing method with MT-COI forward and reverse primers provided.

Sequence and phylogeny analysis: The raw sequence chromatograms were manually checked then assembled and edited using Chromas Version 2.6.5. (Technelysium Pty Ltd, Brisbane, Australia). The identity of the species was checked by homology search in BLASTn search tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for the MT-COI gene. The processed sequences were deposited to NCBI website to obtain accession numbers. The partial sequences showing 99-100 per cent similarity to *H. chenopodii* MT-COI were retrieved from NCBI (MG988841, MW630128, MT021799, MT162454, MT162455 and MG401358) for further analysis. In order to investigate the genetic link, a total of twelve (retrieved 7 no. and generated 5 no.) 435bp MT-COI sequences were aligned using ClustalW after being modified with Bio edit. MEGA X Software (Molecular Evolutionary Genetics Analysis, Version X) was used to carry out the phylogenetic analysis based on the ML statistical approach (Tamura *et al.,* 2013; Kumar *et al.,* 2018). The T92 distance model (Tamura 3-parameter) was used to select the nucleotide mismatch for each region. The Neighbour-Joining (NJ) phylogenetic tree approach was used to create the phylogenetic tree using 1000 bootstrap replications to evaluate the branches' dependability (Saitou and Nei, 1987). Because they are closely related species, brown plant hopper *Nilaparvata lugens* (Accession number: AF222883) sequence from NCBI GenBank was used as an outgroup to illustrate the difference in lineage diversion.

RESULTS AND DISCUSSION

Taxonomic characterization of *H. chenopodii* different life stages:

The eggs are swollen without micropyle, about 0.30 - 0.32 mm long and 0.14 - 0.15 mm broad. The front end of the egg is slightly elevated from the leaf surface and taper to a visible point, with the base of the egg extensively in touch with the leaf surface. Eggs are attached to leaves by a short pedicel (Figs. 2, 7).

Nymph body length varies from 0.7-1.0mm and width varies from 0.4-0.6mm (Fig. 9). When nymphs are in their first instar, they are bright yellow with red ocelli. As they grow, the colour changes to a pale yellowish-green with powdery coating (Figs. 3, 7). Nymphs rarely move and prefer the abaxial leaf surface. The forewing pads were extended anteriorly into humeral lobes. Body covered with setae all around (Fig. 9).

As for adult, both sexes have complete wings. Head and thorax are dark brown to black in mature specimens and yellowish brown in younger specimens (Fig. 4); abdomen yellowish green to darker green (Figs. 5, 6). The length of the adult body can vary from 1.3-1.9mm. Their legs are mostly yellow. Antenna varies in pigmentation, but generally segments II-V pale and the remaining

Fig. 1 Field symptoms of *H. chenopodii* on *C. quinoa* plants, marginal rolls on leaves

Fig. 2 Eggs of *H. chenopodii*

Fig. 3 Nymphal stage of *H. chenopodii*

Fig. 4 Newly emerged adult of *H. chenopodii*

Fig. 5 Matured adult of *H. chenopodii*

Fig. 6 Older adult of *H. chenopodii* with abdominal coloration

Fig. 7 Nymphs emerging from eggs of *H. chenopodii*

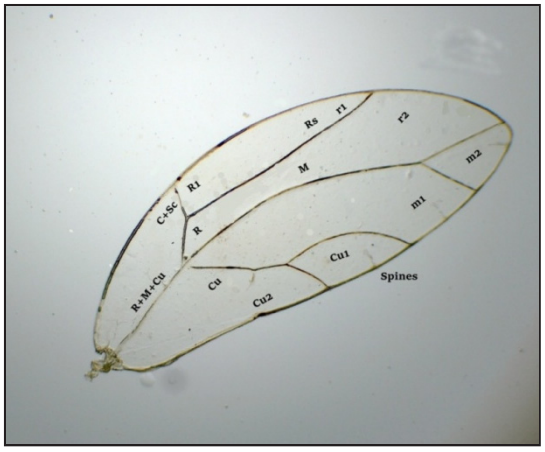

Fig. 8 Wing venation of adult *H. chenopodii*

Fig. 9 Nymphal *H. chenopodii* with sectasetae

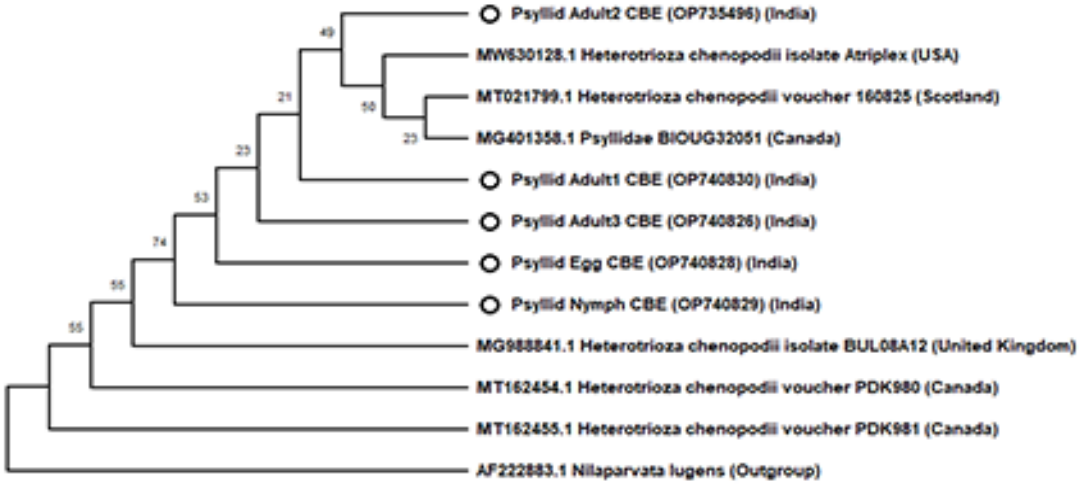

Fig. 10. Dendrogram representation of Neighbour-Joining tree based on 435 bp alignment of 11sequences of the MT-COI gene of *H.chenopodii*. The sequences obtained in this study are indicated in circles

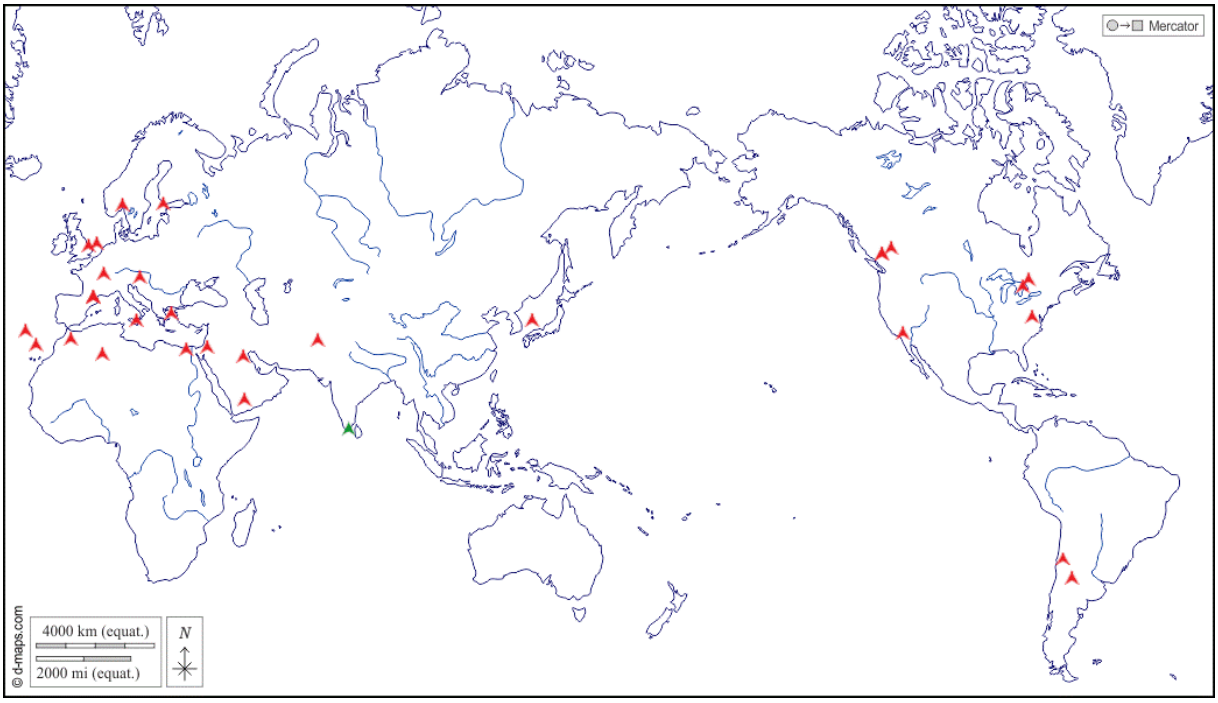

Fig. 11 Graphical display depicting global expansion range of *H. chenopodii*. The red triangles denote the record of *H. chenopodii* from that geographical range. Graphic updated using the reference of Global Biodiversity Information Facility (GBIF)

segments are brown or black.Wings are hyaline with trifurcation of veins (R+M+Cu1) which is typical to Triozidae. The forewing is distinctly broader in the middle, narrowing to an acute apex. Forewing is having a reduced venation and hind wings are filled with minute spinules and with slight brown colour pattern. The vein arising from humeral area (R+M+Cu1), connects the coastal and subcoastal veins with radius. In the forewing, veins Cul and M have a common stem, each arising separately from common origin at vein R (Fig. 8). The apical area of wing contains three apicular spines at median and cubital areas. Forewing with surface spinules largely confined to basal half of wing. Generally abdomen is green in colour for adults. After the last nymphal molt, emerging young adults are initially pale green, but soon the dorsal colorationby maturation become darker with considerable variation in the degree and intensity of colours. In older specimens, dorsum was often found to be uniformly dark brown coloured on the head and thorax, and also on the terminal part of the antennae such colour variations can be seen.

Molecular characterization and Phylogeny:

The identity analysis in NCBI indicated that the MT-COI sequences of *H. chenopodii* were 96 per cent identical to the previously deposited sequences of *H. chenopodii* with NCBI. The generated sequences were further processed and deposited to the NCBI website andaccession numbers assigned were OP735496, OP740826, OP740828, OP740829 and OP740830. The phylogenetic tree shows a single clade of sequences which represented the similarities in the analyzed sequences (Fig. 10). Every sequence had converted into a single clade with 9 subclades excluding the out group. Out of these subclades, eight were with a single sequence and only one subclade was further divided into three. The Indian populations are found to be merged in between other similar populations. *H. chenopodii* is having historical evidences of distribution into sub- continental or continental scales out of their native range (Fig. 11). It was recorded in several countries present in temperate Asia, North Africa, Europe, and the Middle East

(Mifsud, 2020; Percy *et al.,* 2020) and reached the terrain areas of Pakistan too (Wheeler and Hoebeke, 1997, 2013). Now, the insect's geographical range isextended to tropical southern India, on *C. quinoa,* a pseudo-cereal that is highly nutritive and has been cultivated to utilize its foliage. Currently in India, the crop has been gaining attention for human consumption because of its medicinal value as an analgesic, anti-inflammatory and protein supplement (Mujica *et al.,* 2003; Bhargava *et al.,* 2006). Infestation of *H. chenopodii* may affect the productivity ofquinoa and may also other members of the Amaranthaceae, which are seriously infested by this pest in other parts of the globe.

*H. chenopodii*is found to be an effective biological control agent against weeds (Haouas *et al.,* 2021). Hence, a careful assessment of the host plant preference among Amaranthaceae members may be required for further pest risk analysis.The presently reported psyllid has no specific history in this geographical region and sequences of *H. chenopodii* generated are highly similar to the global population. The recent phylogenomic analysis by Percy *et al.* (2018) has grouped the *Heterotrioza* with numerous triozids that are exclusively found in the Austro-Pacific region denoting the eastern range origin of the genus *Heterotrioza*. Hence, it is almost certain that their presence in peninsular India is as the result of recent immigration (Percy *et al.,* 2012; Percy *et al.,* 2020; Burckhardt *et al.,* 2021).

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