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Research Article

Mitochondrial genetic homogeneity of South American leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tamil Nadu, India

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Abstract
The South American leafminer, *Tuta absoluta* is an exotic devastative pest on solanaceous vegetables, including tomatoes, which leads to a cent per cent economic loss in India. The molecular markers assist in assessing gene flow, migratory frequencies, and genetic variety, as well as helping to evaluate the genetic makeup and diversification of an exotic species population to indigenous ones. With this, the present study aimed to investigate the genetic divergence of *T. absoluta* in different districts of Tamil Nadu, India. The study depicted the examination of genetic divergence of *T. absoluta* by aiding amplified region of mitochondrial DNA encoding cytochrome oxidase I (COI) from the *T. absoluta* samples gathered from Coimbatore, Dharmapuri and Dindigul districts of Tamil Nadu. The findings showed that the phylogenetic tree constructed from all sequences of *T. absoluta* acquired from the NCBI (National Center for Biotechnology Information) and BOLD (The Barcode of Life Data System) databases exhibited 99 percent identity and aggregated together into a single clade. . 5Hence, the present study revealed the great genetic uniformity in *T. absoluta* populations in India and corroborates that most of the globe rely on the partial COI gene, evidenced by minimal nucleotide diversity.

Keywords: *Cytochrome oxidase I,* genetic homogeneity, Invasive pest, *Tuta absoluta*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of India's major vegetable crops, accounting for about 10.40 % of the total global production and 18.40% of acreage (NHB, 2018). Tomato production is hampered by biotic stresses, *viz.,* pests and pathogens (Pandey *et al.,* 2017). Under confined and open field conditions, the South American leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is an exotic, neotropical, and incredibly damaging polyphagous pest of tomatoes around the world (Desneux *et al.,* 2011; Sridhar *et al.,* 2014; Santana *et al.,*2019; El-Shafie,2020). *T. absoluta* larvae causes excessive leaf mining and also damages shoot region, tomato fruits, flowers and emerging buds of tomato (Desneux *et al.,* 2010). The apparent lack of management tactics can induce cent per cent economic loss in tomatoes (Campos *et al.,* 2017). The range of *T. absoluta* has expanded dramatically since 2006 to include large swaths of Eurasia, Africa and pockets of the Caribbean region and Central America. (Campos *et al.,* 2017, Santana *et al.,* 2019, Verheggen and Fontus, 2019; Zhang *et al.,* 2021).

T. absoluta was noticed in 2014 in India for the first time around the tomato fields of Karnataka and then it started spreading tremendously to other tomato-cultivating regions of the country (Sridhar *et al.,* 2014; Kalleshwaraswamy *et al.,* 2015; Shashank *et al.,* 2015;

Ballal *et al.,* 2016; Sharma and Gavkare, 2017; Singh et al., 2023). Earlier research has shown that *T. absoluta* can easily achieve detrimental rates in recently invaded areas, regardless of the application of pesticides (Bielza, 2010). In fact, *T. absoluta* is challenging to control because of the protection provided by its habit of mining leaves (Desneux *et al.,* 2010). This pest seems to be more difficult to control due to its strong reproductive potential, quick spread, short developmental period, high survival rate, and pesticide resistance (Roditakis *et al.,* 2015; Biondi *et al.,*2018; Machekano *et al.,*2018). Both biotic and abiotic elements that promote *T. absoluta's* adaption and transmission throughout a large geographic region may positively impact the invasion's success (Cifuentes *et al.,* 2011; Bacci *et al.,* 2019). Determining the genetic makeup and distribution of an exotic population with a local population by employing molecular markers aids in comprehending of genetic variation, migration flows, population isolations and other microevolutionary processes linked with genetic divergence (Ito *et al.,* 2011; Mehrkhou *et al.,* 2021).

Many reports have indicated that as most species move to new habitats, their population genetic structure and molecular diversity shift, as noticed in a few exotic species (Rubinoff *et al.,* 2011; Xia *et al.,*2020; Buj et al., 2022). In this connection, due to its variability, tight maternal genetic inheritance, lack of genetic recombination and suitability for analyzing population genetic makeup and seeking the origin of organism differences, mitochondrial DNA is well suited for these purposes (Shashank *et al.,* 2014; Sarma *et al.,* 2016; Zhan *et al.,*2022). Furthermore, mtDNA is a reliable indicator of differences between and within populations (Margam *et al.,* 2011; Xu *et al.,* 2022). *T. absoluta* has expanded to predominant tomato cultivating regions of India as a progressive invasive species (Shashank *et al.,* 2015, 2016), and there is a lack of reports regarding the prevalence of the genetic diversity of *T. absoluta* in Tamil Nadu. Hence, the present study aimed to determine the genetic homogeneity of *T. absoluta* among three different districts of Tamil Nadu. The invasion of *T. absoluta* must be continuously monitored due to its potential for economic destruction and spread, and it is essential to understand the site or source of a population's entry into a new country. Based on these facts above, mt COI was used to detect the basic knowledge of molecular identification and genetic variability of *T. absoluta* population in Tamil Nadu.

MATERIALS AND METHODS

Study area

An Extensive faunistic survey was conducted to collect immature stages and adults of *T. absoluta* on tomato in different regions of Tamil Nadu, such as Dharmapuri (12.130º N, 78.033ºE), Coimbatore (11.016º N, 76.957º E) and Dindigul districts (10.528º N, 77.745º E) of Tamil Nadu during 2016 to 2018. The immature stages, such as larvae and pupae of *T. absoluta* gathered from the field were taken to the Department of Agricultural Entomology, Tamil Nadu Agricultural Univerisity, Coimbatore and reared for the emergence of the adults. The adult insects collected from the study area were preserved in 70 per cent ethyl alcohol for further studies.

Molecular characterization of tomato pinworm

T. absoluta adults infesting tomato were gathered from different districts of Tamil Nadu *viz.,* Dharmapuri (12.130º N, 78.033º E), Coimbatore (11.007ºN, 76.936ºE) and Dindigul districts (10.528ºN, 77.745ºE). Genomic DNA was extracted from a single newly emerged *T. absoluta* adult from the culture (Department of Agrl. Entomology, Tamil Nadu Agricultural University, Coimbatore) by following the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1987). 200 µl of DNA extraction buffer was used to homogenize an individual sample and at 65°C, incubation was maintained for 1 h. The incubated tubes were taken out of the water bath and kept at an ambient temperature to cool down. Chloroform: Isoamyl alcohol mixture (24:1, v/v) (0.8 volume) was poured into the tubes at equal proportions and mixed gently for 10 minutes by inverting the tubes to get an emulsion. Centrifugation was done at 12,000 rpm for 10 min and the resultant pure water phase was shifted to a new sterile tube. Following this, 0.7 volume of ice-cold isopropanol was poured, inverted gently and stored at -20º C for overnight. Centrifugation at 12,000 rpm was done for 10 min to get the DNA pellet and the supernatant poured out. Then, the DNA pellet can be rinsed with 70 per cent ethyl alcohol. After this, the pellets are allowed to air dry and 20 to 40 µl of Tris-EDTA buffer can be added to dissolve the pellet based on its size and then it was stored under -20°C for further use. The quality of this genomic DNA was assessed by adding 0.8 gram of agarose in 100 ml of 1X TBE (Tris Borate EDTA) buffer and melted in a microwave oven. Soon after cooling, 1 to 2 µl ethidium bromide was taken from the stock (10 mg ethidium bromide / ml H_2O). Then, the prepared mixture was added to a priorly set template having wells made with comb impression. In each well, 2µl DNA mixed with 2 µl loading dye (6X loading dye) was loaded. For 1h at 65 V, Electrophoresis was done. After this, visualization of genomic DNA amplification was done under UV transilluminator (Bio-Rad, USA) and documentation was carried out using Gel documentation system (GELSTAN 1312). In order to quantify the DNA, Nanodrop Spectrophotometer (ND-1000) was employed. Depending on the nanodrop analysis, dilutions of DNA was done using TE buffer and final concentration was made to 50 ng μl^{-1} and stored at 4°C for further studies (Sambrook *et al.,* 1989).

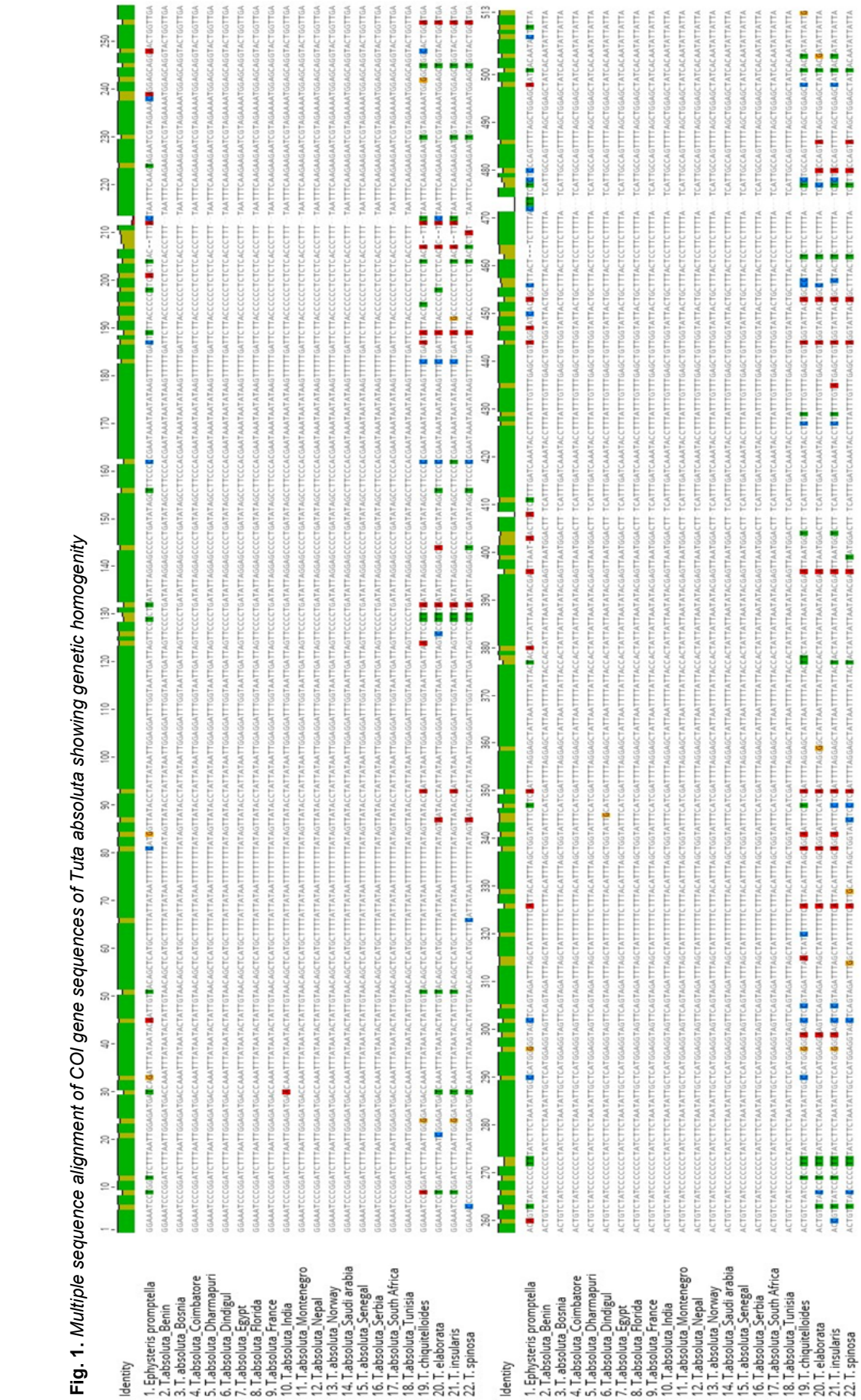
Cytochrome Oxidase 1 (CO1), which is a portion of the mitochondrial gene, was transcribed in leafminers and parasitoids populations by aiding Folmer primers LCOI490 (Forward) and HCO2198 (Reverse) (Hebert *et al.,* 2003). Forward primer (5'-3') : GGTCAACAAATCATAAAGATATTGG, Reverse primer (3'-5') : TAAACTTCAGGGTAACCAAAAAATCA. Polymerase chain reactions were carried out with optimized PCR cocktail mixture (for 25 µl reaction mix) which comprises: 1.5U/ µl of 0.5µl Taq polymerase, 1.0µl of 250 µM of each dNTPs, 2.5µl of 10X PCR reaction buffer consisting of 25mM MgCl, 1.0µl of forward and reverse primers and 16.5µl of nuclease-free water in PCR machine (Sure cycler 8800, Agilent Technologies). The PCR conditions followed were the initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturing for 20 s at 95 °C, annealing for 0.30 sec at 52 °C, and extension time of 40 s at 72 °C, with a final extension for 10 min at 72 °C. Visualization of PCR products was done by employing UV transilluminator on 1.5% agarose gel; further, it was documented in gel documentation system (GELSTAN, 1312). 20 µl of these PCR products along with 10 µl of relevant forward and reverse primers encompassing suitable tags were sent for sequencing to Agrigenome Labs Pvt. Ltd., Cochin, Kerala. Double-pass sequencing was applied to sequence the PCR products both forward and backward orientation. Big Dye Terminator V3.1 Cycle Sequencing Kit was utilized for sequencing PCR products and it was filtered using the Pure Link PCR purification Kit. The resultant sequencing data were fetched from the client database of Agrigenomelabs online portal. The sequences were then edited, clipped, and harmonised using Geneious, and outgroups were retrieved from GenBank using the blastn tool to explore for nucleotide (nr/nt) data base. The nucleotide sequences were compared to determine their degree of similarity between each host by Basic Local Alignment Search Tool (BLAST) and Barcode of Life Database. The gene sequences were aligned using the ClustalW algorithm (Thompson *et al.,* 1994). The phylogenetic tree was built using MEGA version 6.06 and the tree was charted using the neighbourhood joining method.

RESULTS AND DISCUSSION

One of the most destructive pests that threaten tomato crops globally is *T. absoluta*. This insect has expanded throughout most of Europe, Africa, and now Asia in the past ten years, wreaking havoc on tomatoes and their global trade (Campos *et al.,* 2017). Details on genetic divergence and population genetics of an exotic species are crucial to designing invasive species management techniques (Bhattacharya *et al.,*2022). In this scenario, Mitochondrial DNA has been exploited for deducing genetic divergence, identification and origin of invasive species and speciation of cryptic insect species (Hafiz and Samreen, 2016). *T. absoluta* sample populations gathered from various localities of Tamil Nadu, India *viz.,* Dharmapuri, Dindigul and Coimbatore districts and analyzed by aiding standardized mt *COI* gene sequencing method resulted in amplified product length of 680 bp. A GenBank BLAST exploration indicated cent per cent sequence identity with *T. absoluta*. The sequences of *T. absoluta* from Tamil Nadu were submitted to NCBI and Accession numbers for various districts of Tamil Nadu such as Dharmapuri (MN525178), Coimbatore (MN525185) and Dindigul (MN525198) were obtained. The sequences of *T. absoluta* were compared with other *Tuta* spp. obtained from NCBI and BOLD databases. Multiple Sequence Alignment (MSA) of *T. absoluta* did not show any remarkable nucleotide divergence (Fig. 1).The phylogenetic tree of *T. absoluta* constructed with mtDNA (COI) partial sequences using the neighbour-joining method in mega v6.06 is given in Fig. 2. *T. absoluta* from Dharmapuri, Dindigul, Coimbatore, South Africa, Tunisia, Serbia, Senegal, Saudi Arabia, Norway, Nepal, Montenegro, France, Florida, Egypt, Bosnia, Benin and India (Karnataka) were clustered together in a single clade with a bootstrap value of 99 per cent. All these species were closely related to *T. spinosa* from California, with bootstrap support of 62 per cent.

The results are in accordance with Hadapad and Hire (2019), who confirmed the tomato leafminer species available in Telangana, Tamil Nadu, Karnataka, Haryana, Maharashtra and Himachal Pradesh as *T. absoluta* through molecular characterization. Earlier reports have also evidenced the presence of *T. absoluta* in various regions of India by employing *COI* gene (Sidhu *et al.,* 2017; Balaji *et al.,* 2018; Shinthiya *et al.,* 2019). *T. absoluta* sequences that were retrieved from the NCBI and BOLD databases were all grouped together in a single clade and exhibited 99 percent identity in the phylogenetic tree. The current findings noticed considerable genetic homogeneity between different populations of *T. absoluta,* which is apparent by little nucleotide variation. This indicates that *T. absoluta* is conquering new places due to its high reproductive potential, year-round availability of hosts, and a lack of robust phytosanitary precautions during commerce or other logistical means. Ndiaye *et al.* (2021) documented the complete genetic similarity of *T. absoluta* specimens collected regardless of sampling date, geographic area, the host plant, and seasons in Africa. Shashank *et al.* (2018) also noticed significant genetic uniformity in Nepal and Indian *T. absoluta* populations. The mtCOI sequences of seven Tunisian populations of *T. absoluta* indicated the high genetic homogeneity and concluded this as ITS 1, 2 (Asma *et al.,* 2017). High genetic stability was established by the COI sequences of *T. absoluta* populations across the Mediter-

Fig. 1. *Multiple sequence alignment of COI gene sequences of Tuta absoluta showing genetic homogenity*



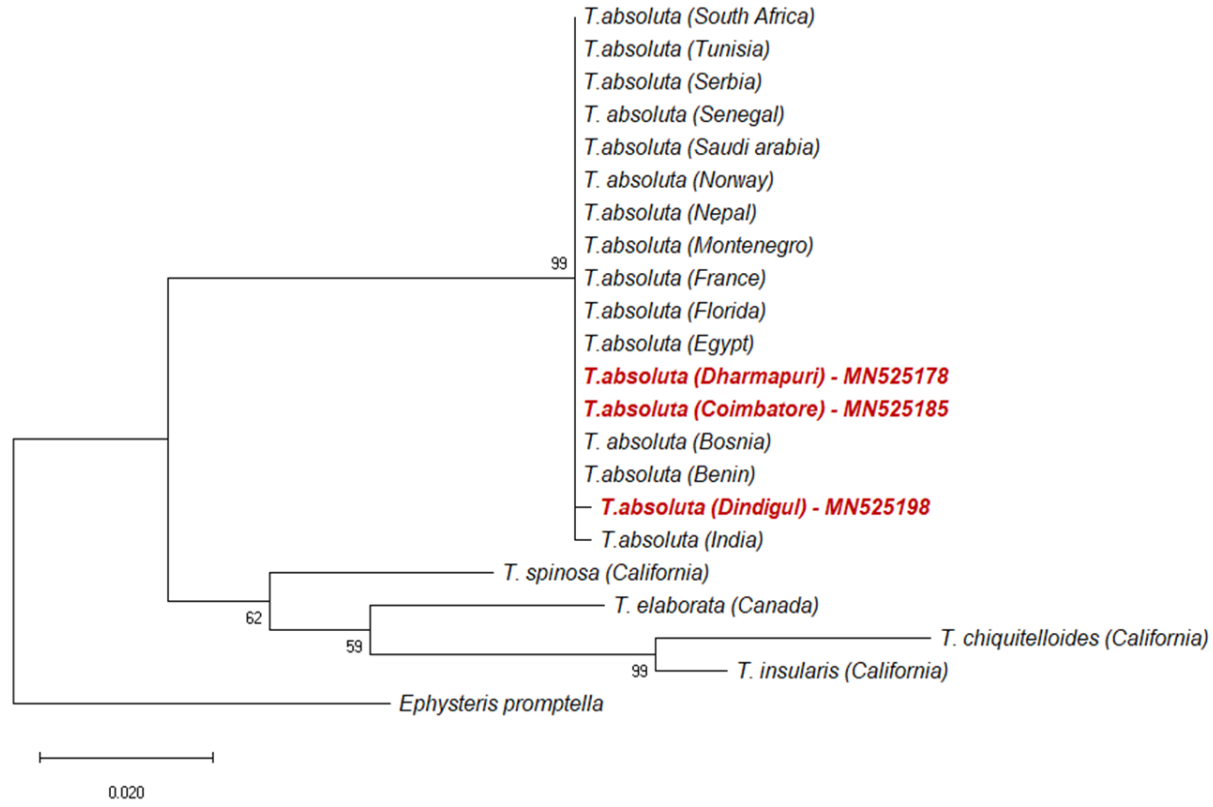


Fig. 2. *Phylogenetic tree of Tuta absoluta obtained from mtCOI gene sequences using neighbour joining method showing genetic homogenity*

ranean Basin and South America (Cifuentes *et al.,* 2011). Other research on *T. absoluta* populations adopting Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique revealed a huge level of genetic divergence as well as notable population heterogeneity in Tunisia (Bettaïbi *et al.,* 2012). Based on microsatellite markers, Guillemaud *et al.* (2015) found that the indigenous South American population of *T. absoluta* is very far from genetic homogeneity and exhibited the origin of the exotic population with the notion of single vs numerous invasions. Genetic uniformity in newly invaded species in a new environment is not unusual; other arthropods have also shown similar accuracy *viz., Linepithema humile* (Tsutsui and Case, 2001), *Sitobion avenae* (Figueroa *et al.,* 2005), *Ochlerotatus caspius* (Porretta *et al.,* 2007), *Cacopsylla pyricola* (Kang *et al.*, 2012), *Oryctes rhinoceros* (Reil *et al.,* 2016), *Drosophila melanogaster* (Zahoor *et al.,*2017) and *Maruca vitrata* (Chatterjee *et al.,* 2019).

Newly introduced populations are less genetically distinguishable than their native population of origin since they are merely a proportion of genetic diversity found in the native population (Cifuentes *et al.,* 2011). Likewise, reduced genetic divergence is apparent in the case of the small amount of introduced populations. Nevertheless, genetic variability suppression is normally deleterious, although in some arthropods, it has benefited to the prosperity of exotic species (Roy *et al.,*2022; Wang *et al.,*2023). Founder effects on the genetic uniformity of Mediterranean communities of *T. absoluta* were proven by Cifuentes *et al.,* (2011). Moreover, mtCOI and rDNA investigations revealed genetic uniformity in two continents (South America and Europe), implying that Mediterranean populations are likely descended from a South American population or that the South American population is invading (Cifuentes *et al.,* 2011).

The invasive behavior of this species and its biological traits could substantially influence the reported genetic homogeneity effect. Genetic uniformity or at least reduced genetic divergence in invading species is not frequent in human-mediated biological invasions, but due to bottlenecks and founder effects during the establishment and colonization phase (Gortat *et al.,*2017). Taxa that have experienced significant bottlenecks or founder effects, causing a decline in genetic diversity have frequently exhibited low mitochondrial DNA variation (Davies *et al.,*2022).

Extreme bottlenecks, either caused by demographic shifts or merely population extension from a limited pool, can potentially wipe out entire mitochondrial lineages or completely wipe out intrapopulation genetic variation in mtDNA, erasing the invasion's history. However, founding events are predicted to dramatically re-

duce genetic variety in invading populations. As a corollary, populations in introduced regions have less genetic variation than the population(s) from where they have origin. Many genetic studies, however, have shown that invasive species do not often exhibit the predicted decline in genetic diversity. Due to the absence of genetic variations in local populations, almost all *T. absoluta* have the same mitotype worldwide. Recent homogenization effects generated by human activities, notably agriculture, elevated rates of naturally occurring or human-mediated gene flow supported by globalization and interconnectedness, or a short evolutionary history of the lineage under consideration may cause in genetic uniformity (Naik *et al.,*2020). If applicable, this should be dealt with by utilising many specimens and extremely quickly changing markers, as genetic homogeneity is never entirely established. Agricultural techniques in *T. absoluta* substantially support widespread gene flows and dispersal. From Africa to Asia, North and South America and Europe, tomato crops particularly, and other solanaceous crops in general offer a comparatively homogeneous habitat.

The path of invasion in Afro-Eurasia and the Middle East implies that *T. absoluta* can spread and colonize new places without the intervention of humankind (Guimapi *et al.,*2020). Both dispersion methods may significantly influence species expansion and gene flow. Surprisingly, the range-wide mitochondrial genetic uniformity of *T. absoluta* has been shown to be quite comparable to that found in parthenogenetic species or cloned animals. Parthenogenesis occurs in certain insects, *viz.,* aphids, bees, wasps and very few lepidopterans, some of which are agriculturally important pests (Liu *et al.,* 2018). This breeding method typically results in declined genetic divergence among the populations investigated, as proven in the case of aphids (Morales-Hojas *et al.,*2020). Earlier research has shown that some *T*. *absoluta* populations have deuterotokous parthenogenesis (*i*.*e*. both sexes are equally produced) (Abbes and Chermiti, 2014; Grant *et al.,*2021). A further plausible scenario involves a recurrent population extinction and colonizations that reduce genuine genetic diversity (Charmouh *et al.,*2022). One scenario that meets this criterion is when *T. absoluta* populations are exposed to local extinction by synthetic pesticides, which is the major strategy for the management of *T. absoluta* in India.

Conclusion

The present study revealed the great genetic uniformity in *T. absoluta* populations in India and corroborates that most of the globe relies on the partial COI gene, evidenced by minimal nucleotide diversity. Due to a general diversity deficit among samples from indigenous and introduced regions, utilizing mitochondrial markers to rectify genetic variations of *T. absoluta* was quite unsuccessful. The present results showed that *T. absoluta* has very low genetic diversity across nearly its complete range, implying a new, quick invasion of a single invasive haplotype with likely several bottlenecks or founder events, proceeded by tremendous growth, resulting in apparent genetic uniformity. Moreover, other genetic markers, such as microsatellites, could be employed in upcoming research to track the actual origin of this damaging invading pest to design effective management techniques.

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Conflict of interest

The authors declare that they have no conflict of interest.

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