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Drawing the Phylogenetic Tree of *Tenothrips* species Using MOLE-BLAST: A Phylogenetic Analysis Approach

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HIGHLIGHTS

- This research employs MOLE-BLAST to build a tree for *Tenothrips* species, focusing on COI / 18S genes.
- MOLE-BLAST aids in identifying closely related sequences for phylogenetic analysis.
- This study improves our grasp of *Tenothrips* species' phylogeny, offering future research insights.

Abstract

The search for the neighboring sequence of the query for the construction of the phylogenetic tree above led to the use of MOLE-BLAST. The construction of the phylogenetic tree was demonstrated using *Tenothrips* species from Muğla, Konya and Antalya in 2023. The *Tenothrips* species were found to show some degree of relationships based on the tree diagram in all regions. The study aimed to draw the phylogenetic tree of *Tenothrips* species using MOLE-BLAST, a computational tool widely used for phylogenetic analysis by COI and 18S Ribosomal RNA gene regions.

Keywords: Neighbor-Joining tree; MOLE-BLAST; *Tenothrips*; COI; 18S Ribosomal RNA

1. Introduction

MOLE-BLAST, serving as a proximity-based exploration tool, facilitates taxonomists in locating the most closely related database neighbour for submitted query sequences. It can be described as a mechanism that imparts taxonomic context by computing multiple query sequence alignments (Altschul et al. 1990) along-side their top BLAST database hits (NCBI 2016). This process uncovers relationships among these sequences and subsequently illustrates the outcome of the neighbourhood search in a phylogenetic tree (Adebule 2018).

MOLE-BLAST is a specific subset of the broader BLAST (Basic Local Alignment Search Tool), designed to identify similarities between biological sequences, whether nucleotide or protein. The tool conducts a comprehensive comparison with sequences in the database, subsequently evaluating the statistical significance of these matches. BLAST's utility extends to the identification of gene family members, leveraging functional and evolutionary associations recorded within the GenBank database (Wolfe et al. 2014).

Distinguishing itself from other BLAST tools, such as smartBLAST (targeting highly similar proteins), primer-BLAST (for PCR primer design), Global Align (comparing sequences across their entirety), and MOLE-

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BLAST stands out. It serves as a valuable resource for taxonomists, ecologists, and other researchers by allowing the submission of sequences to the National Centre for Biotechnology Information (NCBI) to verify the precise taxonomic annotation of these sequences (Adebule 2018). However, it is important to note that one disadvantage of using NCBI databases for sequence data is the possibility of inaccuracies, which could lead to incorrect analysis results. Therefore, researchers should carefully scrutinize and validate the data before conducting analyses.

Furthermore, MOLE-BLAST empowers users to evaluate sequence membership within taxonomy groups, discover neighbouring sequences, visualize the relatedness of reference specimens to sequences, and segment a substantial set of sequences into distinct genes or loci (Adebule 2018). Understanding the evolutionary relationships among species is crucial for unravelling the patterns of biodiversity and comprehending the processes that have shaped life on Earth.

In the context of Thysanoptera, a diverse order of insects commonly known as thrips, investigating their phylogenetic relationships provides insights into their evolutionary history, adaptive strategies, and ecological roles. The genus *Tenothrips* Bhatti, comprising 19 species globally, originates from the Mediterranean region. However, its distribution has expanded to encompass numerous regions worldwide (ThripsWiki 2023). The species within this genus demonstrate associations with a variety of Asteraceae species (Zhang et al 2018). Within the territory of Türkiye, three species of *Tenothrips* have been documented: *Tenothrips discolor* (Karny), *Tenothrips frici* (Uzel) and *Tenothrips anatolicus* (Priesner) (Tunç and Hastenpflug-Vesmanis 2016). In this study was aimed to draw the phylogenetic tree of *Tenothrips* species using MOLE-BLAST, a computational tool widely used for phylogenetic analysis by COI and 18S Ribosomal RNA gene regions. The study conducted by Şahin Negiş and others (2022) focuses on the molecular characterization of species belonging to the Thripidae family, whereas the present study introduces a method for constructing phylogenetic trees of *Tenothrips* species using a tool called MOLE-BLAST. This study specifically highlights the use of MOLE-BLAST for thrips phylogenetic analysis.

2. Materials and Methods

The phylogenetic analysis was conducted using a MUSCLE multiple alignment was computed for MOLE-BLAST. The dataset consisted of one sequence retrieved from NCBI, MW579077 *T. frici*. All *Tenothrips* species were collected from Muğla (Ula/Akyaka[1]), Konya (Karatay/Yarma[2]), and Antalya (Serik[3]) in 2023, following the methodology described by Şahin Negiş et al. (2022). The samples were collected by shaking method on a white plate and transferred into tubes containing 70% ethanol. Sampling was done on many weeds, ornamental plants, and grains. Table 1 shows that sample information including date, coordinates and GenBank accession numbers. In the initial step, a pre-diagnosis was performed under a stereo microscope, and a sample was allocated to represent each region, some for morphological purposes and some for-DNA isolation. The DNA isolation was carried out individually for all specimens using the 'CTAB' protocol developed by Doyle and Doyle (1987). For the mitochondrial Cytochrome Oxidase Subunit, I (COI) gene region (~350 bp), as described by Timm et al (2008), were employed. Additionally, the 18S Ribosomal RNA (~650 bp) primers used were the same as those detailed in Şahin Negiş et al study (2022). Sequencing was performed using the Sanger sequencing method after the PCR stage in both gene regions (COI and ITS) by BM Labosis.

Table 1. The samples information collected during the 2023 in many weeds, ornamental plants, and grains.

Location	Coordinate		Species name	n		Date	GenBank Accession Number	
	N	E		♀	♂		COI	ITS
1	37°03'19.4"	28°20'52.2"	*Tenothrips discolor* (Karny)	47	19	03.09.23	PP537394	PP554182
2	36° 55' 18.56"	31° 6' 4.58"	*Tenothrips frici* (Uzel)	13	2	15.05.23	PP537391	PP554181
							PP537392	-
3	37°48'27.33"	32°53'14.67"	*Tenothrips anatolicus* (Priesner)	42	0	01.08.23	PP537393	PP554183

3. Results and Discussion

In this study, the MOLE-BLAST analysis encompassed all sequences (GenBank accession numbers are given in Table 1) from different gene regions as a unified dataset. One of MOLE-BLAST's notable features is its capability to manage input sequences originating from distinct genes or loci. Faced with such diverse inputs, MOLE-BLAST efficiently clusters them and conducts separate Multiple Sequence Alignments (MSAs) and phylogenetic tree constructions for each locus (NCBI 2023). Additionally, this feature can enable the generation of Neighbor-Joining (NJ) trees for different loci by employing MOLE-BLAST immediately after multiple gene sequencing processes, such as Next-Generation Sequencing (NGS).

The initial phylogenetic analysis encompassed all sequences, including both COI and 18S Ribosomal RNA, and was conducted using MOLE-BLAST in NCBI website. It is an experimental tool meticulously crafted to aid taxonomists in the quest to pinpoint the closest database neighbors for their submitted query sequences (NCBI 2016). And the results were generated separately under distinct locus headings (Figure 1 and 2).

After multiple alignment under locus 1, the NJ tree based on the COI region was constructed using MOLE-BLAST. One GenBank sequence and two *T. frici* samples clustered together on the tree, while the other *Tenothrips* species recorded in Türkiye were in different clades. One of the most well-known barcoding gene regions is Cytochrome Oxidase Subunit I (COI), which serves as the cornerstone of a global bio identification system for insects (Hebert et al 2003). In the MOLE-BLAST tree (Figure 1) made in the study, *T. discolor* species was located close to the *T. frici* species, and this result was parallel to the COI result by Şahin Negiş et al. (2022).

For two of the *T. frici* specimens, there was a strong concordance with the reference specimens for *T. frici*. Similarly, the data for *T. anatolicus* formed another cluster, consistent with the findings of Şahin Negiş et al (2022). Figure 1 demonstrates that *T. discolor* occupies a position nearby but in a distinct clade compared to the other *Tenothrips* species.

Additionally, when examining locus 2 in the multiple alignment, the NJ tree based on the 18S r-RNA gene region revealed that *T. discolor* and *T. anatolicus* shared common branches, while *T. frici* appeared in a separate branch (Figure 2). The same similar branches were observed when analysing the COI gene region.

18S Ribosomal RNA gene sequences have proven invaluable for exploring phylogenetic relationships, finding extensive use in tracing evolutionary history across a broad spectrum (Hillis & Dixon 1991). These sequences are particularly useful for examining relationships among species within the same genus and among closely related genera (Hao et al 2013). Furthermore, the NJ tree based on the 18S Ribosomal RNA gene region, specifically locus 2 in the multiple alignment, revealed that *T. discolor* and *T. anatolicus* share common branches, while *T. frici* forms a separate branch (Figure 2). Besides *T. discolor*, which is in the same branch as *T. frici* taken from the gene bank (KC513013), *T. anatolicus* is located near this branch, which consists of the genus *Tenothrips* (75% bootstrap value) (Şahin Negiş et al 2022). The MOLE-Blast *T. frici* branch formed in the current study is located at an equal distance from both species. Such phylogenetic trees built with the MOLE-BLAST tool should be reconstructed with more samples and broader sequence data, as well as other sequences of the same species and the same gene region in the GenBank.

This analysis showcases the versatility of MOLE-BLAST in handling diverse genetic datasets and highlights its utility in elucidating the phylogenetic relationships among *Tenothrips* species. Understanding these relationships contributes significantly to our comprehension of biodiversity patterns and the processes that have shaped the evolutionary history of this diverse group of insects. Additionally, that kind of a tool can facilitate easier comparison of the obtained data, enhancing the efficiency and precision of genetic analyses.

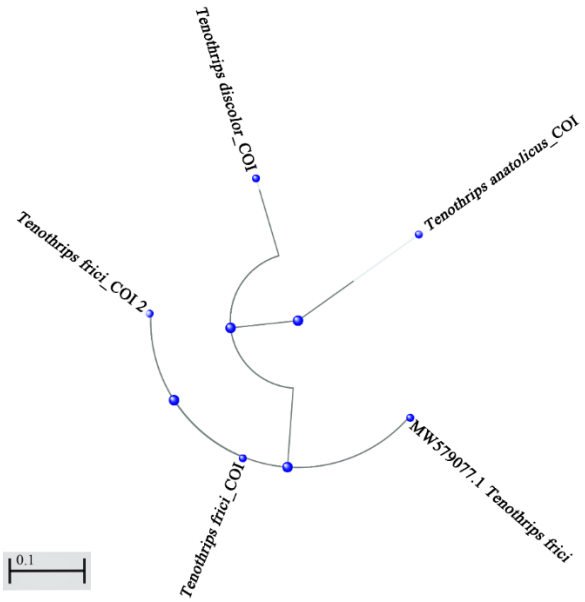

Figure 1. The Neighbor-Joining (NJ) circular phylogenetic tree of *Tenothrips* species, based on the COI gene region, was constructed using MOLE-BLAST.

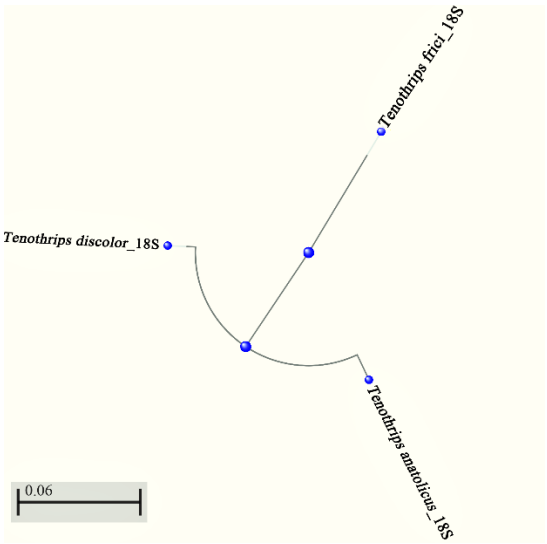

Figure 2. The Neighbor-Joining (NJ) circular phylogenetic tree of *Tenothrips* species, based on the 18S Ribosomal RNA gene region, was constructed using MOLE-BLAST.

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