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Exploration of ITS region as DNA barcode for *Kakothrips priesneri* Pelikan phylogeny

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ABSTRACT

The Thripidae family is a diverse group of insects with global distribution and significant economic importance as pests of agricultural crops. Accurate identification and classification of Thripidae species are critical for their effective management and control. To aid in this effort, DNA barcoding using the ITS gene region has proven to be an efficient and reliable tool for identifying and differentiating Thripidae species. The high variability rate of the ITS region makes it particularly effective for identifying and classifying closely related species, providing valuable insight for understanding and preserving the taxonomic diversity of the Thripidae family. The present study aims to explore the effectiveness of the ITS gene region for DNA barcoding of *Kakothrips priesneri* Pelikan species. For the first time, the ITS data obtained from this study showed the placement of *Kakothrips priesneri* in both common methods, using DNA barcoding (BLAST and MEGA), produced positive results for the ITS analysis, indicating that the region may be suitable for the *Kakothrips* species.

1. Introduction

Thrips (Thysanoptera) are very small insects, with an average length of 1-2 mm, found all over the world (Mehle and Trdan 2012). The order is considered complex due to its high level of homoplasy, which means that different species may have similar characteristics that have evolved independently (Gauld and Mound 1982; Mound and Palmer 1983; Retana-Salazar 1998; 2000). Thrips are divided into two suborders: Terebrantia, which has nine extant families, and Tubulifera (Moritz 1994; Moritz et al. 2000; ThripsWiki 2023). While the Aeolothripidae family is not known to include any pest species (Mound 1997), the Thripidae family, which includes three subfamilies: Panchaetothripinae, Dendrothripinae, and Sericothripinae, has some species that can cause serious crop losses (Mound et al. 2022). Out of approximately 6000 Thysanoptera species identified so far (ThripsWiki 2023), some species are known to be pollinators or biological control agents (Mound and Kibby 1998; Trdan et al. 2005).

The genus *Kakothrips* Williams comprises of eight known species (ThripsWiki 2023). Records of *K. dentatus* Knechtel from Greece (zur Strassen 1996) and Türkiye (Şahin et al. 2019) confirm the Mediterranean affiliation of the genus, despite *Kakothrips dentatus* being primarily distributed in northern Europe (zur Strassen 2003). *Kakothrips acanthus* Berzosa was described by Berzosa (1994) from various locations in Spain and Sicily, and until recently, it was only known from the western and central Mediterranean regions. However, the occurrence of *Kakothrips acanthus* has been documented by Şahin Negiş (2023) in Konya, expanding its distribution range towards the eastern Mediterranean. Among all *Kakothrips* species, females of *Kakothrips priesneri* Pelikan are particularly unique as they possess a pair of pore plates on the third abdominal segment. This species is noted at couplet 6(4) in zur Strassen (2003) as having females with a single pore plate on sternite III, and an image of such a structure in *K. priesneri* is provided by Şahin Negiş (2023). Additionally, *K. priesneri* has longitudinal rows of thickened setae on the tibia (zur Strassen 2003).

Several studies have demonstrated the efficacy of using the ITS gene region for identifying and classifying different species within the Thripidae family. The ITS2 region is a commonly employed DNA barcoding region for identifying Thysanoptera species, as it exhibits sufficient variability among different species. Ashfaq and Hebert (2016) utilized DNA barcoding to investigate Thrips diversity in Pakistan and revealed the existence of cryptic species complexes. Almási et al. (2016) employed the ITS gene region for identifying *Thrips tabaci* Lindeman and determining cryptic species complexes. Tyagi et al. (2017) used DNA barcoding to discover a new cryptic species in *Thrips palmi* Karny in India. These studies highlight the significance of the ITS gene region as a valuable tool in identifying species within the Thripidae family and determining cryptic species complexes.

In this study, two commonly used methods were employed to analyze the DNA barcoding results. The first approach involved using BLAST (Basic Local Alignment Search Tool) to match the query sequences against a reference database and identify the closest match. The second method utilized taxonomic assignment algorithms, such as MEGA, to assign species identities and construct a phylogenetic tree based on sequence alignments. Both methods were applied to analyze both query and *K. priesneri* sequences. The results from both methods were found to be favorable for the ITS analysis, indicating that this region may be suitable for identifying *Kakothrips* species. Furthermore, the

barcode sequence of *K. priesneri* species was uploaded to the GenBank for the first time as part of the present study (accession number OQ779479), contributing to the growing database of genetic information for this species.

2. Materials and Methods

The sample was diagnosed following the method described in zur Strassen (2003). DNA isolation was carried out using the CTAB protocol, with each sample processed individually according to the method described by Doyle and Doyle (1987). The ITS F/R primers, which amplify a region of approximately 650 bp, were used for PCR amplification of the ITS gene region, and the PCR reaction and cycling conditions were the same as those described in Şahin Negiş et al. (2022) (Fig. 1). Sanger sequencing was performed at BM Labosis. After obtaining the sequencing results, each sample was individually analyzed using the Neighbor-Joining (NJ) and UPGMA (unweighted pair group method with arithmetic mean) methods in the Mega11 program. The Kimura 2-parameter model, as proposed by Kimura (1980), was used for calculating genetic distances and time of divergence between sequences. An outgroup sequence, from GenBank accession number MW1865502.1 for *Limenitis archippus* (Cramer) (Lepidoptera: Nymphalidae), was used in the phylogenetic tree as a reference for comparison.

3. Results and Discussion

Thysanoptera, a group of insects commonly known as thrips, presents challenges in taxonomic and phylogenetic studies. Some researchers have argued against relying solely on molecular data for taxonomic decisions in Thysanoptera, as highlighted by Mound and Morris (2007), and have considered it an insufficient solution for addressing taxonomic issues, as noted by Lee (2004). However, recent studies have shown promising results in using molecular techniques for defining species in Thysanoptera. Further research, that compares morphological and molecular data, may help establish more confidence in the use of molecular techniques for taxonomic purposes in this group. Recognition of invasive species is crucial for effective management, and it is important that the process is rapid, cost-effective, technically accessible, and accurate, as emphasized by Darling and Blum (2007). Molecular techniques, such as DNA barcoding, can provide a valuable tool for identifying invasive species, as they can be applied to a wide range of samples and can yield accurate results with relatively small sample sizes. Combining molecular data with other taxonomic approaches, such as morphological and ecological data, can enhance our understanding of invasive species and aid in their management and control.

The utilization of BLAST and taxonomic assignment algorithms in this study allowed for a thorough analysis of the DNA barcoding results for *Kakothrips priesneri* Pelikan. The BLAST method, which compares query sequences to a reference database, and the taxonomic assignment algorithms (MEGA), which assign species identities and generate a phylogenetic tree, were both applied to analyze the query and *K. priesneri* sequences. The positive results obtained from both methods (BLAST and MEGA) in the ITS analysis indicate that the ITS gene region may be appropriate for identifying *Kakothrips* species. Additionally, the barcode sequence of *K. priesneri* was uploaded to the GenBank for the first time, with the accession number OQ779479, contributing to the growing genetic database for this species. These findings highlight the importance of utilizing multiple methods for DNA barcoding analysis, as it allows for a comprehensive and reliable identification of species. The combination of molecular techniques, such as DNA barcoding, with traditional taxonomic approaches can greatly enhance our understanding of species identification, phylogenetics, and biodiversity studies.

The BLAST program was used in this study to search the reference database using the ITS sequence of *Kakothrips priesneri* as the query sequence. The top BLAST hits for the query sequence were mainly from *Frankliniella* species, with a similarity of 97.5%. Other hits with similarity ranging from 92-97% were from various other thrips species. This indicates that the query sequence of *K. priesneri* showed high similarity to *Frankliniella* species, which was expected based on previous knowledge. However, it is also important to note that there were hits with slightly lower similarity to other thrips species, which could potentially indicate close genetic relationships or shared genetic regions among thrips species. The use of BLAST in this study allowed for the comparison of the query sequence against a reference database, providing valuable information on potential matches and similarities with other known thrips species. However, further analysis and verification using additional molecular and morphological techniques would be necessary to confirm the taxonomic identification of *K. priesneri* and related species.

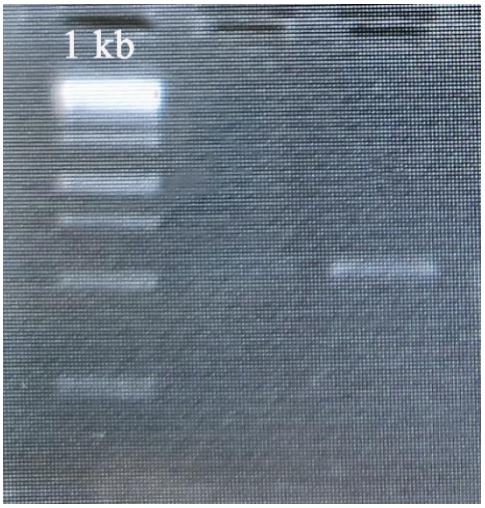

Figure 1. The amplification of *Kakothrips priesneri* in the ITS gene region is approximately 650 bp (Marker 1 Kb).

In this study, the UPGMA (unweighted pair group method with arithmetic mean) and NJ (Neighbor-Joining) methods (Schlee 1975) were used to infer the evolutionary history of 27 nucleotide sequences, with the ITS gene region being the focus of analysis were used . The Maximum Composite Likelihood method (Tamura et al. 2004) was used to calculate evolutionary distances, and all ambiguous positions were removed for each sequence pair using pairwise deletion option, resulting in a final dataset of 603 positions. MEGA11 (Kumar et al. 2016) was used for all evolutionary analyses. The overall mean distance (OMD), which represents the average evolutionary distance between all taxa, was calculated to understand the structure of the phylogenetic tree. OMD is a metric that ranges between 0 and 1, with values close to 0 indicating low genetic differences between taxa, suggesting that the phylogenetic tree accurately reflects the relationships between taxa. In the case of the ITS gene region, a value of 0.03 between taxa is considered to reflect a good relationship in the phylogenetic trees, indicating that the analysis results are reliable in terms of the ITS gene region. This information provides insights into the robustness and accuracy of the phylogenetic analysis conducted in the study, indicating that the relationships between taxa in the phylogenetic tree are well-supported by the ITS gene region data, as reflected by the OMD value of 0.03. The phylogenetic trees constructed in this study were based on the available sequences of *Frankliniella* species and other thrips species from the GenBank database, as there were no sequences available for *K. priesneri* or other *Kakothrips* species. This was done because *K. priesneri* is morphologically similar to *Frankliniella* species (zur Strassen 2003), and thus *Frankliniella* sequences were used as a proxy for inferring the evolutionary relationships of *K. priesneri*.

In both the UPGMA (Fig. 2) and NJ trees (Fig. 3), the outgroup was placed outside the main tree. However, there were some differences observed between the two trees. For example, in the NJ tree, *Parabaliothrips* sp., which is characterized by its abdominal ctenidia terminating at the median lateral seta on tergites VI-VII and pronotum posteroangular setae positioned upper side the posteromarginal setae (OzThrips 2023), was positioned closer to the outgroup compared to the UPGMA tree. This suggests that the evolutionary relationship of *Parabaliothrips* sp. is slightly different when analyzed using the NJ method compared to the UPGMA method. Similarly, in the NJ tree (Fig.2), two species belonging to the genus *Aptinothrips* were observed to be close to each other, while they were positioned far apart in the UPGMA tree (Fig. 3). This indicates that the evolutionary relationship between these two species may vary depending on the method used for phylogenetic analysis. These differences in the positioning of species in the phylogenetic trees may be attributed to the different algorithms used in the UPGMA and NJ methods, as well as the evolutionary distance calculation method (Maximum Composite Likelihood) used in this study. It highlights the importance of using multiple methods and considering different factors when interpreting phylogenetic trees, and the need for further studies with more sequences and additional molecular markers for a comprehensive understanding of the evolutionary relationships among thrips species, including *K. priesneri*.

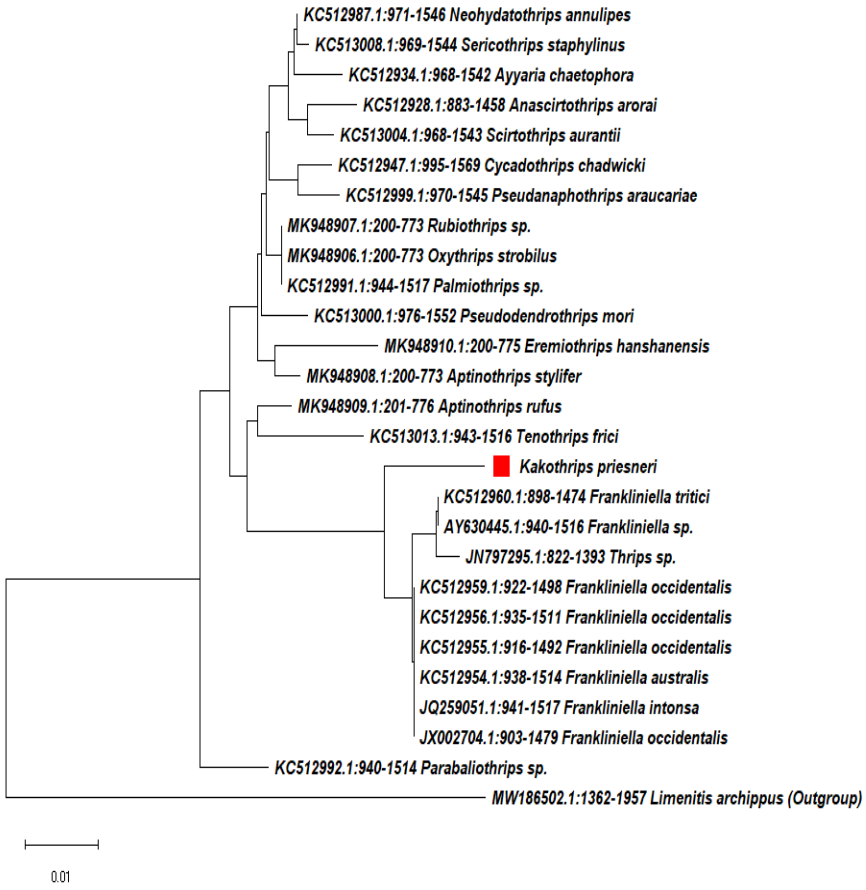

Figure 2. The *Kakothrips priesneri* NJ phylogenetic tree according to the ITS gene region of 27 nucleotide sequences studied with MEGA11. *Limenitis archippus* (Cramer) was used as an outgroup. (Overall mean distance: 0.03).

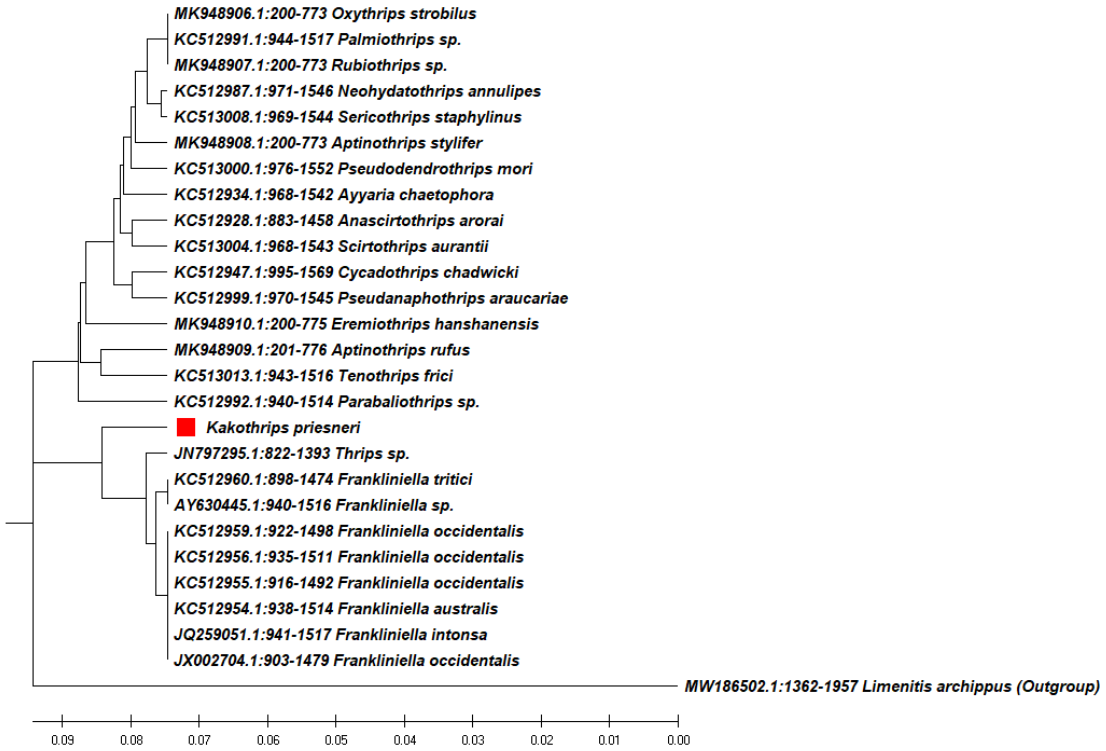

Figure 3. The *Kakothrips priesneri* UPGMA phylogenetic tree according to the ITS gene region of 27 nucleotide sequences studied with MEGA11. *Limenitis archippus* (Cramer) was used as an outgroup. (Overall mean distance: 0.03).

The study suggests further evaluation of the ITS gene region for distinguishing thrips species, as it is important to test it on a larger number of thrips species, particularly those that are phylogenetically distant and dissimilar, in both phylogenetic trees where thrips species are separated. Additionally, the study by Şahin Negiş et al. (2022) reported that the ITS gene region was effective in distinguishing thrips species at the genus level, indicating its potential for higher taxonomic classifications. Therefore, a greater number of thrips species and expanding the taxonomic coverage in the phylogenetic analysis using the ITS gene region may provide more comprehensive information about this gene region and its utility in thrips systematics, classification, and identification.

4. Conclusions

The previous studies have documented the presence of certain thrips species, specifically those belonging to the Thysanoptera family Thripidae, including the newly identified *Kakothrips priesneri* Pelikan species, ITS gene region sequencing in this study. Morphological diagnosis was also implemented to validate the findings. Furthermore, the sequence data was compared with the GenBank references detailed in the figures. The result of this study supports the use of ITS DNA barcode primers for diagnosis. Notably, this study also marks the inclusion of *K. priesneri* species sequence data in GenBank (OQ779479) for the first time. Future studies should focus on expanding thrips sequences in a well-designed network for accurate and robust diagnosis.

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