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Exploring *Aeolothrips* spp. Diversity: A Morpho-Molecular Examination of *Aeolothrips collaris* and *Aeolothrips intermedius* (Thysanoptera: Aeolothripidae)

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ABSTRACT

This study investigated the evolutionary relationship between *Aeolothrips collaris* Priesner, 1919 and *Aeolothrips intermedius* Bagnall, 1934 within the Thysanoptera: Aeolothripidae family. Notably, *A. collaris* DNA barcode was made for the first time and compared with other *Aeolothrips* species in GenBank. While revealing a close genetic association (82-83%) between these species, the analysis using the Neighbor-Joining method clustered *A. collaris* with *A. albicinctus* Haliday, 1836 and *A. ericae* Bagnall, 1920, while *A. albicinctus* grouped alongside *A. fasciatus* (Linnaeus 1758) and *A. duvali* Moulton, 1927 GenBank BlastN analysis supported the expected placement of *A. intermedius.* These insights highlight significant genetic connections but suggest the necessity for a broader examination involving more species and gene regions. Expanding this research could yield a comprehensive understanding of the intricate taxonomic relationships within this thrips genus, setting the groundwork for future investigations into thrips species evolution and taxonomy.

1. Introduction

The order thrips (Thysanoptera) encompasses around 6000 species worldwide, classified into two suborders: Terebrantia, consisting of 2606 species, and Tubulifera, which includes 3809 species (ThripsWiki 2024). Thrips are tiny insects, inconspicuous due to their slender bodies, typically measuring 1-3 mm in length. Their adult stage is defined by narrow, elongated wings adorned with long fringes along the edges, hence the name Thysanoptera, derived from fringed wings (Riley et al. 2018). Thrips exhibit diverse biology and inhabit various environments, engaging in activities from plant feeding and predation to consuming fungi. Their two pairs of well-developed wings are positioned outside the thrips' body. Additionally, they possess piercing-sucking mouthparts. One distinctive trait of thrips is the asymmetry in their mouthparts, where only the left mandible is present (Parker et al. 2013). Their short antennae typically consistis of VI to X segments, depending on the species (Loomans et al. 1995). Aeolothripids are identified by their broad forewings, typically rounded at the apex and often displaying shaded, colorless transverse bands. Additionally, their antennae consist of nine segments, with segments III-V elongated and parallel-sided, featuring flat, linear, or oval-shaped sensoria on antennal segments III and IV. Moreover, their ovipositor is upturned (Mirab-Balou et al. 2011). The Aeolothripidae family, belonging to the suborder Terebrantia, comprises approximately 240-250 species exhibiting a wide array of feeding behaviors. Among many species within this family, both adults and larvae act as facultative predators, preying on other small arthropods. They feed not only on floral tissue but also on thrips and mites residing within flowers (Conti 2009; Parker et al. 2013).

Thrips, through their feeding on developing flowers or vegetables, frequently become pests affecting commercial crops. Additionally, they can act as carriers of plant diseases such astospoviruses (Reitz 2009; Ullman et al. 2007). Incorrectly identifying an economically significant species can have far-reaching and serious consequences, leading to confusing data across different biological disciplines. Accurately identifying thrips species stands as the initial and crucial step in gathering genetic and other biological data necessary for effective management strategies. Morphological traits such as color, chaetotaxy, and body structure primarily underpin thrips species identification. However, their small size, secretive behavior, sexual differences, developmental stage similarities, and polymorphism (seen in color, wing development, body size, etc.) present hurdles for morphology-based identification. Given these challenges, it becomes essential to employ supplementary methods for thrips species identification and resolving taxonomic issues. Molecular tools, increasingly utilized over the past decade, have proven valuable in augmenting various biological fields, spanning from systematics to ecology (Alves-Silva and Del-Claro 2010; Tyagi et al. 2017).

Aeolothrips collaris Priesner, 1919 (Thysanoptera: Aeolothripidae) (*A. collaris*) is widely distributed, spanning from southern Europe across the Mediterranean to Madeira and the Canary Islands, further extending eastward to encompass regions in India and Bangladesh. It has also been introduced to California (Alavi and Minaei 2018; zur Strassen 2003). Additionally documented in Iran, China, India, France, Albania, Egypt, Cyprus, the Canary Islands, Mongolia, Türkiye, and Bangladesh (Mirab-Balou 2013; Tunç 1991). *Aeolothrips intermedius*

Bagnall, 1934 (Thysanoptera: Aeolothripidae) (*A. intermedius*) exhibits a widespread distribution across both Eastern and Western Europe, as well as in Iran and China (Loomans et al. 1995; Trdan et al. 2005).

1.1. Taxonomy

The two thrips suborders, Terebrantia and Tubulifera, have 9 extant families and 6 fossil families. Among these, Terebrantia comprises 8 extant and 5 fossil families, while Tubulifera includes 1 extant and 1 fossil family (ThripsWiki 2024). In the suborder Terebrantia, the family Merothripidae comprises of 5 genera with 18 species, Melanthripidae includes 6 genera with 75 species, Aeolothripidae consists of 28 genera with 250 species, Fauriellidae encompasses 4 genera with 5 species, Stenurothripidae involves 12 genera with 24 species, and Heterothripidae contains 7 genera with 24 species. Within the family Thripidae, the subfamily Panchaetothripinae contains 40 genera with 140 species, Dendrothripinae includes 15 genera with 100 species, Sericothripinae comprises of 3 genera with 145 species, and the subfamily Thripinae comprises of 240 genera with 1700 species. Uzelothripidae includes 1 genus with 1 species. In the suborder Tubulifera, within the family Phlaeothripidae, the subfamily Phlaeothripinae contains 375 genera with 2820 species, while the subfamily Idolothripinae consists of 80 genera with 715 species (Parker et al. 2013).

1.2. Host plants and feeding behavior

Aeolothrips collaris is often found dwelling among flowers on diverse plants without any noted specificity. It's believed to be a facultative predator, displaying a diverse diet that includes pollen and larvae from other thrips (Priesner 1964).

Aeolothrips intermedius primarily preys on Thysanoptera, comprising 44 species such as *Thrips tabaci* Lindeman 1889 (Thysanoptera: Thripidae), *Frankliniella* spp, and other *Thrips* spp. Additionally, it targets various other prey, including spider mites, psyllid larvae and eggs, and microlepidoptera (Loomans et al. 1995; Trdan et al. 2005). Despite being a predator, *A. intermedius* exhibits a diverse feeding behavior, from leaf cells and petal cells to pollen, expanding its diet across various areas (Parker et al. 2013).

This study aimed to make a comprehensive exploration of *Aeolothrips* diversity, merging intricate morphological assessments with precise molecular analyses. The focal points were the species *A. collaris* and *A. intermedius*. A significant milestone was achieved with the pioneering creation of the DNA barcode for *A. collaris*, enabling subsequent comparisons with related sequences in GenBank. This integrated approach not only revealed the fascinating interplay between morphological variations and genetic makeup within *Aeolothrips* but also laid the groundwork for further taxonomic advancements. The creation of *A. collaris*' DNA barcode stands as a pivotal contribution, promising enhanced insights into the genus' taxonomy and evolutionary patterns.

2. Materials and Methods

In 2021, specimens of the species were collected from Isparta and Konya provinces of Türkiye. Collection involved shaking plants on a tray, and the specimens gathered were temporarily preserved in small vials containing a mixture of 70% ethanol, stored at +4°C. These specimens were later mounted in Hoyer's medium for microscopic examination. The slides carrying the specimens are archived in the Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya, Türkiye. Subsequent to the diagnostic phase, each specimen underwent DNA isolation using the 'CTAB' protocol developed by Doyle and Doyle (1987). For the mitochondrial Cytochrome Oxidase Subunit, I (COI) gene region, the COI deg F1/R1 primers were employed, targeting a segment of approximately 350 base pairs (Timm et al. 2008). The PCR protocol mirrored that used in (Şahin Negiş et al. 2022). The taxonomic distances of the assembled specimens were manually corrected using the MEGA11 analysis program. Each species' gene region was individually aligned, and before tree analyses, overall mean distances were computed using MEGA11 to calculate the mean pairwise distances between taxa. Additionally, *Ixodes ricinus* Linnaeus (Acari: Ixodidae) was used as an outgroup for the phylogenetic tree.

3. Results

3.1. Morphological diagnosis

Aeolothrips collaris and *A. intermedius* are two distinct species of thrips found across different geographical regions and exhibiting notable differences in various morphological characteristics.

Aeolothrips collaris typically displays an antenna segment III that is predominantly yellowish white, with a brown to dark brown coloration near the apical edge. The antennal segment III is typically yellow, with brown coloring in the apical fifth or less. Segment V measures approximately 1.2 times the length of segments VI-IX combined (Fig. 1b). Moreover, the antennal segment IV is usually 4.0-4.2 times as long as its width. The pronotum exhibits a range from yellow to dark brown. Fore legs are usually lighter than the mid and hind legs (Fig. 1a). The dark transverse band on the fore wing tends to be about 1.12-1.28 times as long as it is wide (Fig. 1c). In some instances, the prothorax and abdominal segments III and IV might have a yellowish hue. Females of this species measure between 1790-2125 µm (zur Strassen 2003; Alavi and Minaei 2018). The abdomen displays marginal setae on sternites that originate either at or near the margin. Sternite VII specifically features two pairs of accessory setae arising notably in front of the margin. In males, tergites IV and V exhibit paired dorsal tubercles. Additionally, on tergite IX, the setae located at the base of the claspers are shorter than the clasper itself, accompanied by a stout curved seta positioned laterally to the clasper (Priesner 1964).

The species *A. collaris* and *A. intermedius* both belonging to the Thysanoptera: Aeolothripidae, are closely related within the *Aeolothrips* genus. Interestingly, these two species showcase both similarities and differences, which provide valuable insights into their characteristics and relationship. However, according to (zur Strassen 2003), *A. collaris* is considered synonymous with *A. intermedius*, indicating a shared identity between the two species. The comparative analysis of sampled specimens and sequence comparisons among closely related species uncovered substantial patterns in evolutionary relationships, offering insights into the identities and connections within this group.

On the other hand, *A. intermedius* has approximately three or four generations per year. Adult insects begin to appear in April and May. Females begin laying eggs after emergence, and the duration of egg laying ranges from two to four weeks. The female lays 29-73 eggs, and the incubation period for the eggs lasts for

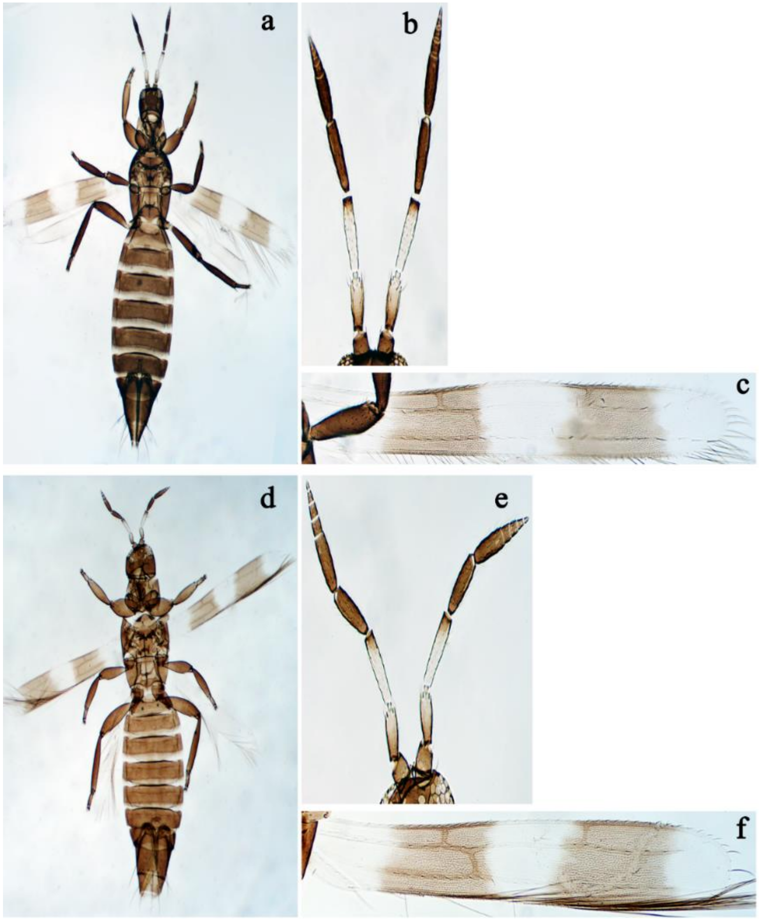

Figure 1. *Aeolothrips* female species. *A. collaris* (a-c): **(a)** body, **(b)** antenna, **(c)** right wing. *A. intermedius* (d-f): **(d)** body, **(e)** antenna, **(f)** right wing.

21 days at a temperature of 14, 6 days at a temperature of 26, and 4 days at a temperature of 38 (Loomans et al. 1995)**.**

Aeolothrips intermedius demonstrates an antenna segment III that starts as light yellow or yellowish brown but darkens notably from the middle or at least in the apical third towards the dark brown apical edge. Antennal segment IV, in this species, tends to be 3.1-3.8 times as long as its width. Antennal segment III ranges from yellow to brownish-yellow, gradually darkening to brown in the apical fourth to half. Segment V is about the same length as segments VI-IX combined (Fig. 1e). The pronotum is consistently brown. Fore legs are not lighter than the mid and hind legs (Fig. 1d). The length of the fore wing's distal dark band at the anterior margin is 1.0-1.5 times the length of the pale area between the dark bands. Its distal dark transverse band on the fore wing is typically 1.26-1.43 times as long as it is wide (Fig. 1f). The prothorax is consistently dark, and females measure between 1850-2400 µm (zur Strassen 2003; Alavi and Minaei 2018).

3.2. Molecular diagnosis

The generation of *A. collaris*' DNA barcode and its comparison with sequences in GenBank showcased a close genetic association among related species, underscoring the potential for utilizing DNA barcoding in future taxonomic studies within the genus. These findings emphasize the significance of combining multiple analytical methods to unravel the intricate relationships and evolutionary trajectories within *Aeolothrips*, setting a solid foundation for continued research in thrips taxonomy and evolution.

Utilizing the BlastN option with the *A. collaris* type, *Aeolothrips* sp. displayed identities ranging between 82-83%. Additionally, sequence matches revealed percentages of 76-77% with *Aeolothrips albicinctus* Haliday, 1836 (*A. albicinctus*), 80% with *Aeolothrips duvali* Moulton, 1927 (*A. duvali*) (which had only two sequence options), 82% with *A. intermedius*, and 79-80% with *Aeolothrips ericae* Bagnall, 1920 (*A. ericae*) and *Aeolothrips fasciatus* (Linnaeus 1758) (*A. faciatus*).

The Neighbor-Joining method (Saitou and Nei 1987), was employed to infer the evolutionary history, presenting the optimal tree. Bootstrap analysis (500 replicates) demonstrated the percentage of replicate trees wherein associated taxa clustered together, with values indicated alongside branches (Felsenstein 1985). Using the p-distance method (Nei and Kumar 2000), evolutionary distances were calculated in units of base differences per site. The analysis encompassed 42 nucleotide sequences, considering codon positions 1^{st}+2^{nd}+3^{rd}+ noncoding. Ambiguous positions were eliminated for each sequence pair using the pairwise deletion option. The final dataset comprised 290 positions. Evolutionary analyses were performed using MEGA11 (Tamur et al. 2021). In the NJ tree, *A. collaris* clustered closely with *A. albicinctus* and *A. ericae*, while *A. intermedius*, *A. faciatus* and *A. duvali* species clustered together. As confirmed by the GeneBank BlastN percent identity (100%) result, the *A. intermedius* sample took its place in the tree together with the GeneBank sequence samples of the same species (Fig. 2).

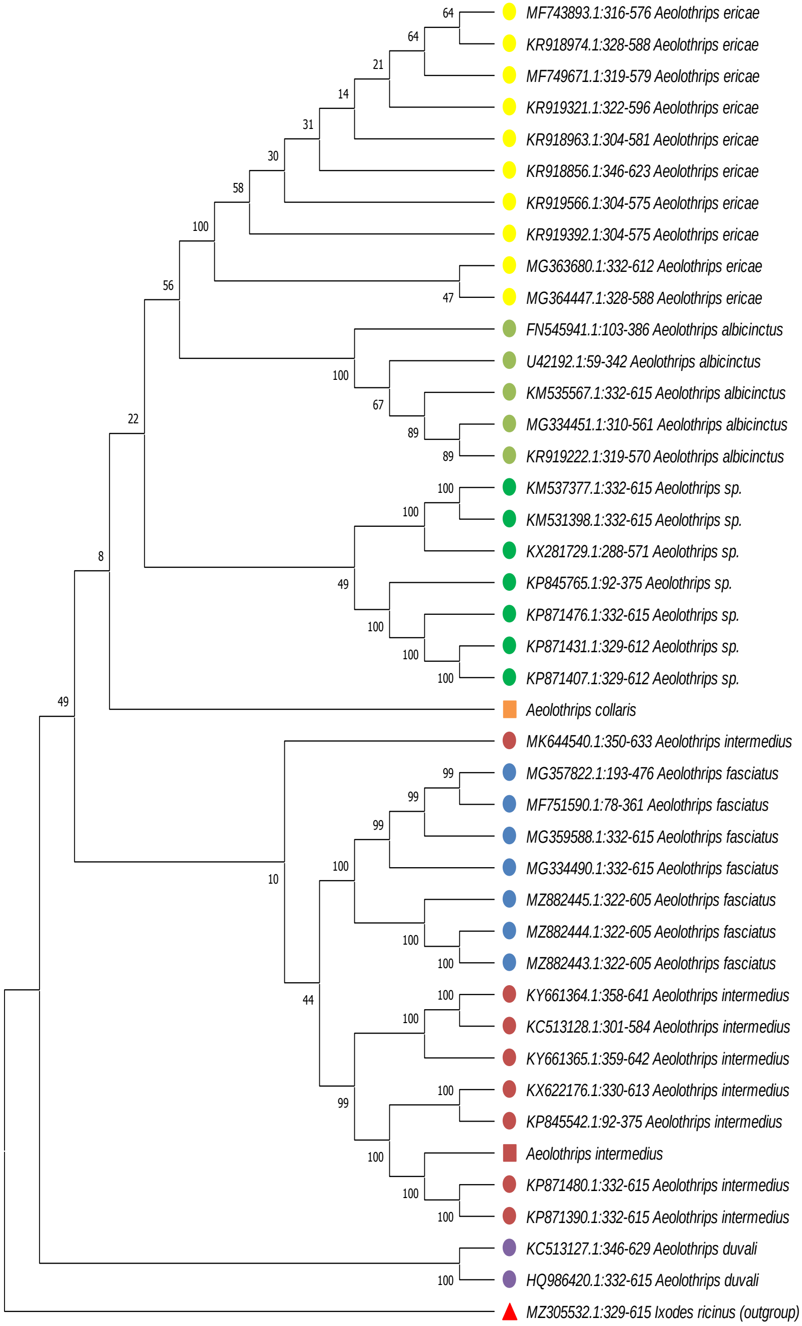

Figure 2. The NJ phylogenetic tree was constructed based on the COI gene region of *A. collaris* and *A. intermedius* species. *Ixodes ricinus* Linnaeus (Acari: Ixodidae) was utilized as an outgroup (MZ305532.1). The numbered sample sequences were obtained from GenBank. (Overall mean distance: 0.20).

In the NJ tree illustrating branching from the outgroup root species, *A. albicinctus*, *Aeolothrips ericae, A. intermedius, A. fasciatus*, *A. duvali*, and *A. collaris* species exhibit a shared ancestral positioning. Upon examining the resulting tree, it is suggested that *A. collaris, A. albicinctus, Aeolothrips sp.*, and *A. ericae* might share a closer relationship and a common ancestor. Further sampling is anticipated to enhance the comprehension of evolutionary relationships, potentially clarifying similarities or differences among species. With more extensive sampling and analysis using genetic data, it is anticipated that studies will facilitate a clearer understanding of relationships among species and their evolutionary history.

The *Aeolothrips* genus, a substantial subset within the Arthropoda class, demonstrates notable variations among its species, particularly in distinct anatomical features. Detailed examinations among species like *A. albicinctus, A. ericae, A. intermedius*, and *A. collaris* revealed morphological disparities, especially in abdominal coloration patterns, prothorax characteristics, and tonal variations in antennal segments. These pronounced discrepancies play a pivotal role in species identification and classification.

Distinctive features, such as the dark body with specific abdominal patterns in *Aeolothrips albicinctus*, or the coloration and length differences in antennal segments in *Aeolothrips ericae*, offer crucial markers for species differentiation. Furthermore, characteristic variations in prothorax coloration and antennal segment shades between *A. intermedius* and *A. collaris* serve as prominent identifying factors. Additionally, specific sensory structures on abdominal tergite I distinguish *A. fasciatus* from other *Aeolothrips* species (zur Strassen 2003).

4. Discussion and Conclusion

The findings of this study open avenues for substantial discussion. The amalgamation of morphological and molecular analyses provided a holistic perspective on *Aeolothrips* diversity, particularly focusing on *A. collaris* and *A. intermedius*. The creation of *A. collaris*' DNA barcode, a notable first, served as a valuable tool for comparative genomic analyses, affirming close genetic affinities among certain *Aeolothrips* species. However, while shedding light on these species' genetic associations, the study also underscored the necessity for broader sampling across additional species and genetic regions to fortify taxonomic assessments. Moreover, the integration of molecular data with traditional morphological taxonomy accentuates the significance of a multidisciplinary approach in elucidating thrips diversity. This study's implications stretch beyond *Aeolothrips* alone, advocating for a more comprehensive understanding of thrips evolutionary dynamics and taxonomic frameworks. Future endeavors encompassing an expanded dataset and varied methodologies are crucial to unravel the complexities of thrips taxonomy and evolution, offering a clearer lens into the vast world of these tiny yet evolutionarily significant insects.

The distribution of the Aeolothripidae family displays an inherent asymmetry, evident both in its taxonomic structure and geographic spread. While a substantial 55% of described aeolothripid species find their primary habitat within the largely Holarctic confines, notably encapsulated within the *Aeolothrips* and *Melanthrips* genera, an equally noteworthy statistic emerges: 50% of the acknowledged genera within this family are distinctly tropics-bound (Mound and Marullo 1998) and, the feeding habits of both species are similar, and according to (House 1966), the consumption of various prey items and potentially plants might influence the behaviors of flower-dwelling predators such as *A. intermedius*, a point of particular significance. Additionally, adults of *Aeolothrips intermedius* require feeding on flowers to reach sexual maturity. In the absence of prey, a floral diet can sustain complete larval development in the predatory insect. It's emphasized that *A. intermedius* is primarily a predator of thrips (Bournier et al. 1979).

By examining and contrasting these distinct morphological features among species, a clearer understanding of their classification and identification within the *Aeolothrips* genus emerges. These findings contribute significantly to the broader understanding of evolutionary relationships and species differentiation within this taxonomic group.

While both species belong to the *Aeolothrips* genus and share habitats within the herbaceous and shrub layers, they differ in characteristics such as the coloration and proportions of antennal segments III and IV, the presence of yellow hues on specific body parts, the length-to-width ratio of the dark transverse band on the fore wing, and the size range of females. These distinctions help distinguish them from one another and aid in their taxonomical classification and ecological understanding.

This study examined the evolutionary relationship between *A. collaris* and *A. intermedius* within the *Aeolothrips* genus. While indicating a close genetic relationship (82-83%) between these species, the analysis using the Neighbor-Joining method revealed the clustering of *A. collaris* with *A. albicinctus* and *A. ericae,* whereas *A. intermedius* grouped with *A. fasciatus* and *A. duvali*. GenBank BlastN analysis supported the anticipated position of *A. intermedius*. These findings, while highlighting significant genetic connections, underscore the necessity for a more extensive study encompassing a broader array of species and gene regions. Expanding this research could provide a comprehensive understanding of the intricate taxonomic relationships within this thrips genus and lay the foundation for future investigations into the evolution and taxonomy of thrips species.

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