Selcuk Journal of Agriculture and Food Sciences
SJAFS
http://sjafs.selcuk.edu.tr/sjafs/index
(2022) 36 (2), 203-211
e-ISSN: 2458-8377
Research Article
DOI:10.15316/SJAFS.2022.026

Molecular Characterization of Thripidae (Thysanoptera) Species in Karaman, Konya and Mersin (Turkey)

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ARTICLE INFO

Article history:
Received date: 21.05.2022
Accepted date: 07.06.2022

Keywords:
Barcoding
COI
18S
Sanger
Thripidae

ABSTRACT

Specimens of Thripidae (Thysanoptera) species collected from Konya, Karaman and Mersin (2015-2017) were studied through molecular analyses as a part of preliminary attempts to establish a barcoding system for Thysanoptera in Turkey. The analyses included 23 species namely; *Chirothrips kurdistanus* zur Strassen, *Chirothrips manicatus* Haliday, *Frankliniella intonsa* (Trybom), *Frankliniella occidentalis* (Pergande), *Frankliniella tenuicornis* (Uzel), *Limothrips angulicornis* Jablanowski, *Odontothrips confusus* Priesner, *Odontothrips dorycnii* Priesner, *Oxythrips ajugae* Uzel, *Taeniothrips inconsequens* (Uzel), *Tenothrips anatolicus* (Priesner), *Tenothrips discolor* (Karny), *Tenothrips frici* (Uzel), *Thermothrips mohelensis* Pelikan, *Thrips angusticeps* Uzel, *Thrips atratus* Haliday, *Thrips italicus* Bagnall, *Thrips linarius* Uzel, *Thrips major* Uzel, *Thrips meridionalis* (Priesner), *Thrips physapus* Linnaeus, *Thrips tabaci* Lindeman and *Thrips trehernei* Priesner which were detected in various cultivated plants and weeds. The phylogenetic positions of the 9-genera, with few exceptions, to form their own clades by the 18S Ribosomal RNA data on the NJ tree. The 18S data were discussed for the first time for *Th. linarius, Th. angusticeps, Th. italicus, Th. meridionalis, Th. physapus, F. tenuicornis, Ch. kurdistanus, Te. anatolicus, Te. discolor, Ther. mohelensis* and *Ox. ajugae* species. Clearly results from the COI dataset in a single UPGMA tree separated the genera. The COI data of *Li. angulicornis, Te. anatolicus, Od. confusus, Od. dorycnii Th. angusticeps, Th. atratus, Th. italicus, Th. linarius* and *Th. meridionalis* were obtained for the first time by this study.

1. Introduction

Thysanoptera or thrips are characterized by having of a prolongable sac-like arolium at the top of each leg and by their asymmetric 'punch and suck' mouthparts in which the left side mandible only is developed (Heming, 1971; 1978). Scarcely 1 % of the described species thrips are potential pests (Mound and Teulon, 1995) but also, they have pollination, predation, and natural enemies opportunuties for agriculture (Mound, 2005). Thysanoptera is divided into two suborders; Terebrantia and Tubulifera and has approximately 6,337 extant species in 786 genera. Terebrantia is classified into seven families, Uzelothripidae, Merothripidae, Aeolothripidae, Adiheterothripidae, Fauriellidae, Heterothripidae and Thripidae. Thripidae with more than 2100 species, is the second largest family of Thysanoptera and most of the pest thrips, and all of the tospovirus vectors, belong to this family (Wiki, 2020). Although, the most known viral disease is *Tomato spotted wilt virus* (TSWV) but 26 different diseases caused by tospovirus by thrips transmission are recorded. (Rotenberg et al., 2015). Meanwhile the total number of thrips species recorded in Turkey from 193 (Tunç and Hastenpflug-Vesmanis, 2016) to 196 with three recent additions by Şahin et. al. (2019), Elekçioğlu (2020), Atakan and Pehlivan (2021), and out of these 116 species are from Thripidae. Though many species, that were reported as pests elsewhere, were also recorded in Turkey, however, among these, currently, only around 14 species are 'documented or potential pests' in this country as *Frankliniella intonsa* (Trybom), *Frankliniella occidentalis* (Pergande), *Thrips tabaci* Lindeman etc.

Polymorphism, lack of solid morphological characters in some cases, coexistence of different species in the same host plant, and high intraspecific variations, bring the need for taxonomic expertise (Murai and Toda,

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2001; Brunner et. al., 2004; Mound, 2012; Kadirvel et. al., 2013).

In addition, systematic status of the genera and species is changed by making the distinction later. On the other hand, early detection and correct and immediate identification of the pest species which stand in the quarantine list (Glover et. al., 2010; Smith-Pardo and O'Donnell, 2015) is very crucial. Furthermore, a simple, accurate, general, and easily applicable method is required in the integrated pest management (Rebijith et al., 2014). Molecular diagnosis seems a promising method to address the problems encountered in morphological diagnosis (Immaraju et. al., 1992; Brødsgaard, 1994; Zhao et. al., 1995; Karadjova, 1998; Kontsedalov et. al., 1998; Jensen, 2000; Espinosa et. al., 2002; Rugman-Jones et. al., 2010).

DNA barcode is considered to be the most effective molecular approach in distinguishing cryptic species, biotypes, haplotypes and host-geographic genetic differences in sex and polymorphism. Besides having such advantages over traditional (morphological) taxonomy, DNA barcode is also assumed to be fast and cost-effective in data collection and analysis stages (Shufran et. al., 2000; Brunner et. al., 2004; Asokan et. al., 2007; Borisenko et. al., 2009; Glover et. al., 2010; Strutzenberger et. al., 2011; Rebijith et. al., 2012; Zhang et. al., 2012; Rebijith et. al., 2013). Nevertheless, DNA barcoding approach is not designated as a competitor to traditional taxonomy in the future, but as the formal protocol for the identification of insects and other groups and as a powerful tool to aid in the discovery and identification of new species (Jinbo et. al., 2011; Leite, 2012).

The present study was carried out to assist this method, which has been used for many organisms in recent years, to what extend would be reliable to establish a barcode system for Turkish Thysanoptera fauna. Thripidae specimens collected in Konya, Karaman and Mersin (Turkey) between 2015 and 2017, were studied using conventional and molecular taxonomy (DNA barcoding method).

2. Materials and Methods

Specimens of Thripidae (Thysanoptera) species collected from Konya, Karaman and Mersin (2015-2017) were studied through molecular analyses as a part of preliminary attempts to establish a barcoding system for Thysanoptera in Turkey. The specimens were collected by shaking plants on a tray and were preserved temporarily in small vials containing a mixture of 60% ethanol+glacial acetic acid (9: 1), later were transferred to vials containing permanent preservation fluid, 70% ethanol, and stored at + 4°C. Specimens were mounted either in Canada Balsam or in Hoyer's medium for microscopical examination. The protocol given by Mound and Kibby (1998) was followed for mounting in Canada Balsam. Specimens were kept in a mixture of phenol+lactic acide (1:1, weight) overnight before mounted in Hoyer's medium. Identification of specimens was carried out by İrfan Tunç. A Motic BA310 high resolution digital camera was used for photomicrography. Terminology follows zur Strassen (2003). Slide-mounted specimens are deposited in the Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya, Turkey.

Following the pre-diagnosis step, the 'CTAB' protocol developed by Doyle and Doyle (1987)was applied individually to all specimens for DNA isolation. The COI deg F1/R1 primers were used for the mitochondrial Cytochrome Oxidase Subunit I (COI) gene region (~350 bp) (Timm et al., 2008). The 18S Ribosomal RNA primers (~650 bp) F (GGTGAA-ATTCTTGGAYCGCGCAAGAC) and R (CGCG-TGCRGCCCCRGACATCTAAG) were designed by examining available sequences from Thripidae taxa deposited at GenBank. PCR reaction was consisted of 0.15 mM dNTP, 2.5 mM MgCl2, 2.5 µL reaction buffer solution, 0.75 units of Taq DNA polymerase (all fermantes) and 0.2 mM each primer, 1 µL of DNA per sample in a final volume of 25 µL. The PCR cycling conditions consisted of an initial denaturing step at 94°C for 150 s, followed by 35 cycles of 94°C for 50 s, 53°C for 50 s, and 72°C for 70 s for the COI amplification. On the other hand, an initial denaturing step at 95°C for 120 s, followed by 35 cycles of 95°C for 90 s, 60°C for 75 s, and 72°C for 75 s was applied for 18S rDNA amplification. After amplification of both gene region, PCR products were sent to Macrogen Inc for sequencing in both directions.

A single sequence (using forward and reverse sequence information) was obtained from 23 species in 9 genera in the COI and the 18S ribosomal RNA gene regions. They were assembled and manually corrected their taxonomic distances using Mega X analysis program. Each gene region and each species were individually aligned within themselves. Before trees analyses, the overall mean distances correlated using Mega X analysis program, which is calculation model to mean all individual pairwise distance between taxa. Each gene was analysed using one of the distance-based UPGMA (Unweighted Pair Group Method with Arithmetic) and NJ (Neighbor Joining) analysis methods (respectively COI and the 18S ribosomal RNA gene regions). The trees were made using the Kimura 2-parameter model (Kimura, 1980), the ratio change between positions is modeled by the gamma distribution (Shape parameter = 1), the software MEGA (X version) to align sequences for all thrips specimens and build the trees of both gene region variants using p-distances using 1000 bootstrapping iterations as suggested by Kumar et al. (2018). *Haplothrips distinguendus* (Uzel) (suborder Tubulifera) and *Aeolothrips ericae* Bagnall (suborder Terebrantia) were used as outgroups for phylogenetic trees, respectively as suggested by Crespi et al. (1996) and Brunner et al. (2002).

3. Results and Discussion

Twenty-three species in 9-genera were detected namely, *Chirothrips kurdistanus* zur Strassen, *Chirothrips manicatus* Haliday, *Frankliniella intonsa* (Trybom), *Frankliniella occidentalis* (Pergande), *Frankliniella tenuicornis* (Uzel), *Limothrips angulicornis* Jablonowski, *Odontothrips confusus* Priesner, Odo*ntothrips dorycnii* Priesner, *Oxythrips ajugae* Uzel, *Taeniothrips inconsequens* (Uzel), *Tenothrips anatolicus* (Priesner), *Tenothrips discolor* (Karny), *Tenothrips frici* (Uzel), *Thermothrips mohelensis* Pelikan, *Thrips angusticeps* Uzel, *Thrips atratus* Haliday, *Thrips italicus* Bagnall, *Thrips linarius* Uzel, *Thrips major* Uzel, *Thrips meridionalis* (Priesner), *Thrips physapus* Linnaeus, *Thrips tabaci* Lindeman and *Thrips trehernei* Priesner (Figure 1) in the present study.

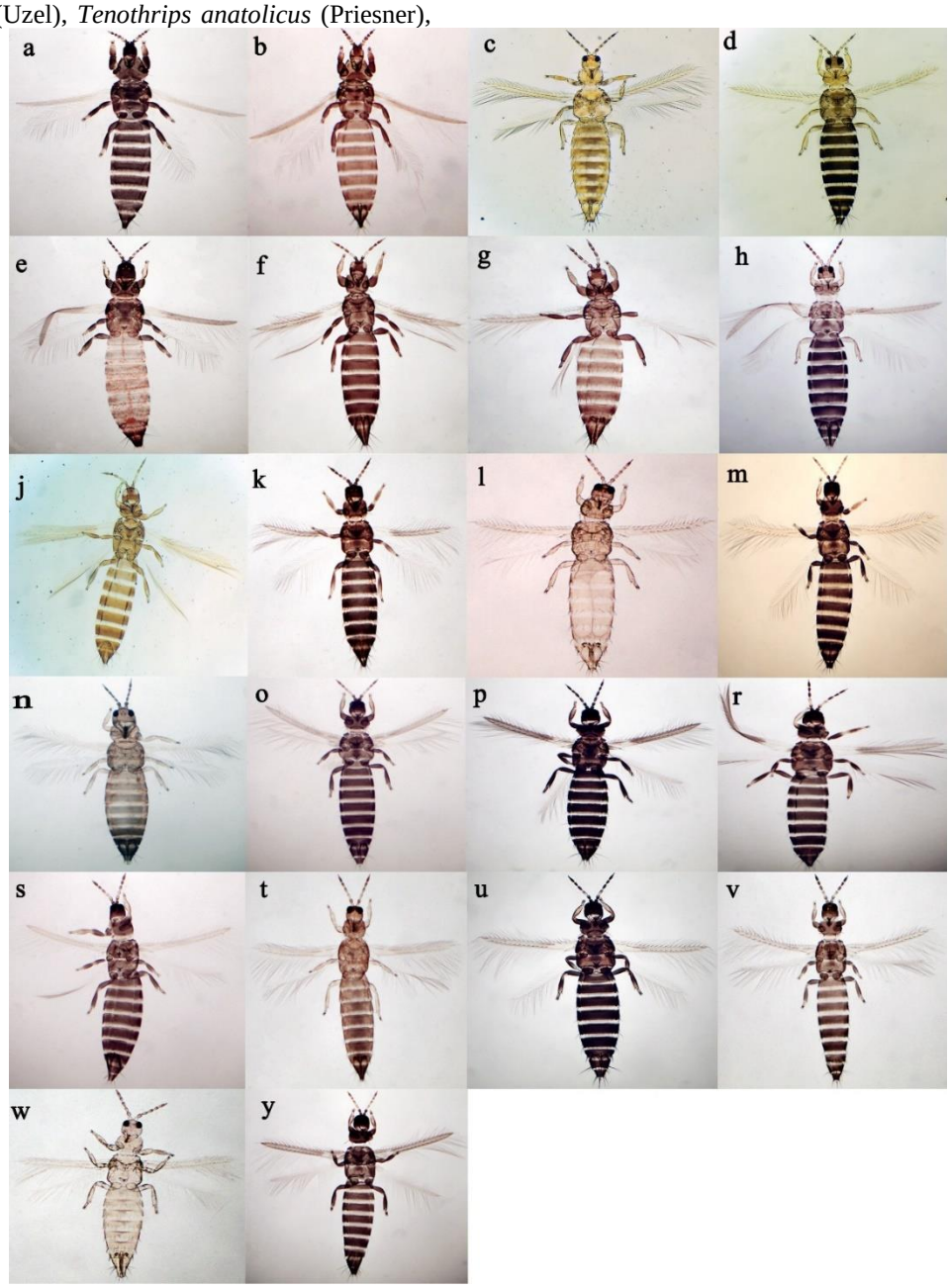

Figure 1
Slide photomicrographs of thrips species studied, all females a.*Chirothrips kurdistanus* b.*Chirothrips manicatus* c.*Frankliniella occidentalis* d.*Frankliniella intonsa* e.*Limothrips angulicornis* f.*Odontothrips confusus* g.*Odontothrips dorycnii* h.*Oxythrips ajugae* j.*Taeniothrips inconsequens* k.*Tenothrips anatolicus* l.*Tenothrips discolor* m.*Tenothrips frici* n.*Thermothrips mohelensis* o.*Thrips angusticeps* p.*Thrips atratus* r.*Thrips italicus* s.*Thrips linarius* t.*Thrips major* u.*Thrips meridionalis* v.*Thrips physapus* w.*Thrips tabaci* y.*Thrips trehernei.*

The phylogenetic positions of the 9-genera with two outgroups observed almost, with few exceptions, to form their own clades by the 18S Ribosomal RNA data on the NJ tree (Figure 2). *Chirothrips* sp., *Th. atratus* and *Te. frici* species were missing from the sequence library and therefore not represented in the tree. The 18S Ribosomal RNA overall mean distance among the species showed a value of 13% which means that mean 18S rDNA data divergence is 13% between the species.

Of the eight species in Thrips taxa appeared in same clade and these data were discussed for the first time for *Th. linarius*, *Th. angusticeps*, *Th. italicus*, *Th. meridionalis* and *Th. physapus* species. For three of the species in *Frankliniella*, there were good agreement from the reference specimens and the data was showed for the first time for *F. tenuicornis* species. However, the two species (*F. intonsa* and *F. occidentalis*) clades were not included the same reference specimens.

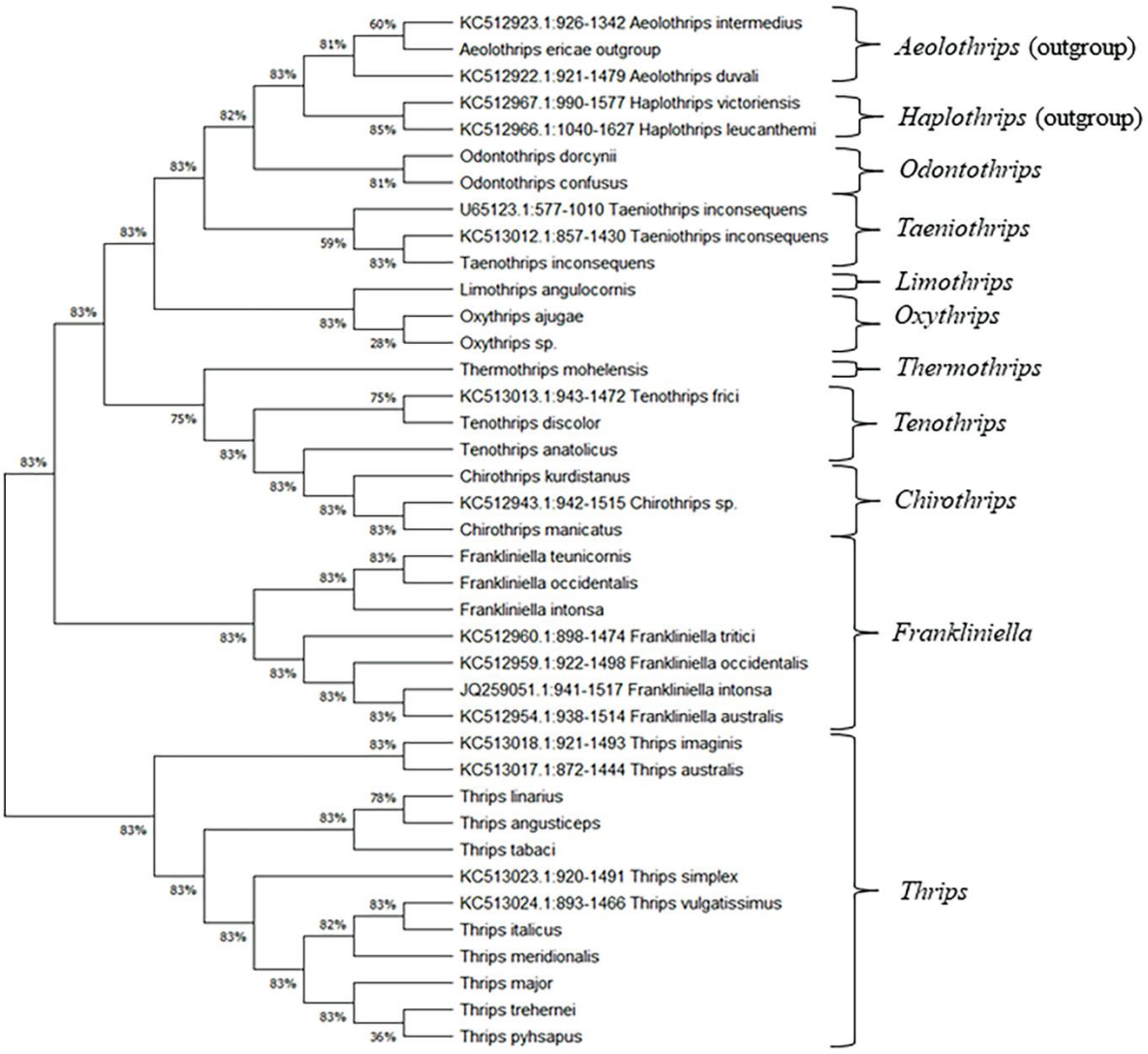

Figure 2
The NJ phylogenetic tree according to the 18S Ribosomal RNA gene region of Thripidae species studied with Mega X. *Aeolothrips ericae* Bagnall was used as outgroup. Sample sequences with numbers were taken from GenBank. (Overall mean distance: 0.13).

Of the two species in *Chirothrips*, recorded in one clade with the reference specimen (KC5122943.1 *Chirothrips* sp.), at the same time that was first time to represent for 18S Ribosomal RNA data of *Ch. kurdistanus*. By contrast *Te. anatolicus* species, which was in the first time for its 18S data, formed an unsupported clade, included *Chirothrips* species. In addition, *Tenothrips discolor* recorded in one clade with the reference specimen (KC513013.1 *Te. frici*) and in the first time for its 18S data in present study. *Thermothrips mohelensis*, the only species of the genus *Thermothrips* (zur Strassen, 2003), was detected for the first time in 18S Ribosomal RNA gene region. Its clade recorded with *Tenothrips*. And also, for first time in 18S rDNA data for *Oxythrips ajugae* and *Oxythrips* sp. species which were classified in a clade, but their clade included in one species *Limothrips*. Of the one species *Taeniothrips* clade recorded together with the two reference specimens. For the two species in *Odontothrips* recorded in one clade and for both are for the first time for 18S data.

18S Ribosomal RNA gene sequences have provided a well of information about phylogenetic relationships and have been used to make out phylogenetic past across a very wide aspect (Hillis and Dixon, 1991) and especially use to examine relationships among congeneric species and closely related genera (Hao et. al., 2013). Just as Hsieh et al. (2020) observed that their 16S mini-barcode system had resolution for species identification universality. Whereas Glover et. al. (2010) stated that when they aligned the ITS2, which is shorter and easier amplifying part of ITS (Hao et. al., 2013), there was unrelated thrips species sequence. Because, as they mentioned that if the ITS2 sequences of only a few species has aligned, it can be completely unrelated. Also, varied studies reported that ITS2 is not more variable than the other parts (van Herwerden et. al., 1999; Tkach et. al., 2000; Vilas et. al., 2005). However, our data came from 18S ribosomal gene region and Kuzoff et. al. (1998) stated that it has a ubiquity and conservative rate of evolution. Because of that the small-subunit ribosomal RNAs (18S) have proved useful for eukaryotes phylogenetic surveys, especially useful for inferring distant phylogenetic relationships between lack of any morphological characters (Sogin et. al., 1972; Woese, 1987; Field et. al., 1988; Turbeville et. al., 1991).

Clearly results from the COI dataset in a single UPGMA tree separated the 9-genera (Figure 3). *Frankliniella occidentalis*, *F. tenuicornis* and *Oxythrips* sp. species were missing from the sequence library and therefore not represented in the tree. The COI overall mean distance among the species showed a value of 24% which means that mean COI sequence divergence is 13% between the species. *Frankliniella intonsa* was observed within the references from NCBI in first clade shortly after the outgroup. There seemed that *Limothrips*, *Thermothrips* and *Oxythrips* species were represented within same clusters together. In addition, all *Oxythrips* were positioned in the same clade with the reference of (MK659499.1) *Anaphothrips* same as those 40 genera of *Anaphothrips* genus-group are recorded in world genera- which one is *Oxythrips*, *Anaphothrips* and *Thermothrips* by Masumoto and Okajima (2017) in their study. *Oxythrips ajugae* was formed with the reference of *Ox. ajugae* (FN546031.1) in the same clade.

The COI data of *Limothrips angulicornis* was obtained for the first time by this study and the species recorded in further clade than *Chirothrips* species cluster although *Chirothrips* and *Limothrips* have been placed together in a group called the Chirothripini (Mound, 2011). Moreover, they are quite different, for instance *Limothrips* has one pair of long postero-angular pronotum setae, when *Chirothrips* has two; the *Limothrips* maxillary palps segmented two, while *Chirothrips*'s three etc. (zur Strassen, 2003), but both of them called grass-living thrips by Mound (2011). One of our unknow species *Chirothrips* sp. was located between reference MF748558.1 *Ch. manicatus* and our sample in the tree. This case suggests it could possibly be in the *Chirothrips manicatus* species-group as reported by Minaei and Mound (2010).

For three of the species in *Tenothrips*, there were good agreement from the reference specimens for *Tenothrips frici* and *Te. discolor*. *Tenothrips anatolicus* data was obtained for the first time for COI gene region and the species was represented another cluster but shortly after with the two *Tenothrips* species. Of the one species in *Taeniothrips* appeared in same clade with the reference specimens. Bhatti (1967) submitted *Tenothrips* as a subgenus of *Taeniothrips* by referring to its trapezoidal pronotum and the basisternum flange of its prothorax with distinct anterolateral lobes. Morphologically, *Tenothrips* and *Taeniothrips* are almost identical in their 8-segmented antennae, Tergite VIII without ctenidium and brown color. However, *Tenothrips* always has two pairs of ante-ocellular setae on the head while *Taeniothrips* species has only one pair (zur Strassen, 2003). And also, as mentioned by Mound (1981) *Taeniothrips* genus-group has not included *Tenothrips* because of some morphological characters. Secondly Bhatti (2003) suggested that *Tenothrips* shares many characters and there are no obvious morphological differences with *Ceratothrips*, a genus of the *Megalurothrips* genus-group. Therefore, diagnosis of it and related genera needs extensive study according to Zhang et. al. (2019). Despite all these studies and different characters, between *Tenothrips* and *Taeniothrips* relationship was talked in the title of *Taeniothrips* genus-group study (Wang et al., 2020).

A total of two of *Odontothrips* species recorded in one cluster with the reference specimens and for the first time for COI data in the literature. Moreover, there is no very big differences between their morphology and also no big molecular difference was observed, only one base in their COI data (data not shown). When we looked at their morphological characters, *Od. confusus* is just larger species than *Od. dorycnii* for example, in terms of the setae S_1 on tergite IX (respectively >140 µm and 90 µm), the hind tibia (respectively 210 µm and 178 µm), body length (respectively 1720-2060 µm and 1430-1650 µm) etc. (zur Strassen, 2003).

The Thrips representing nine species (and five NCBI references) formed distinct 3 clades in a different place from *Frankliniella* genus and also their genus-groups, are not closely related (Mound, 2002). Similarly, Karimi et. al. (2010) indicated that *Thrips* species formed different cluster but stood close together themselves in the phylogenetic tree. The COI data of *Th. angusticeps*, *Th. atratus*, *Th. italicus*, *Th. linarius*, and *Th. meridionalis* were recorded for the first time in present study. *Thrips meridionalis* recorded in a clade with the reference of *Th. vulgatissimus* same as Mound and Masumoto (2005) pointed out the close morphological similarity of them. For instance, there is only little difference about their color of antennal segment III, their length of antennal segments VII and VIII (zur Strassen, 2003). And also, another morphological similarity Thrips including *Th. trehernei* to *Th. physapus*, which was indicated the similarity by Mound and Masumoto (2005), located in a same clade. Both *Thrips* species has only a few morphological characters; *Th. trehernei* abdominal segment X mostly longer than 80 µm (75-95 µm) and its dorsal longitudinal split taking up only 70-85% of the segment length, while *Th. physapus* abdominal segment X mostly shorter than 80 µm (58-82 µm) and it's split occupying 86-95% of segment length. *Thrips major* and *Th. tabaci* formed in the same clade with their reference specimens. The *Chirothrips* representing three species formed innermost one clusters but distinct 3 clades in the tree.

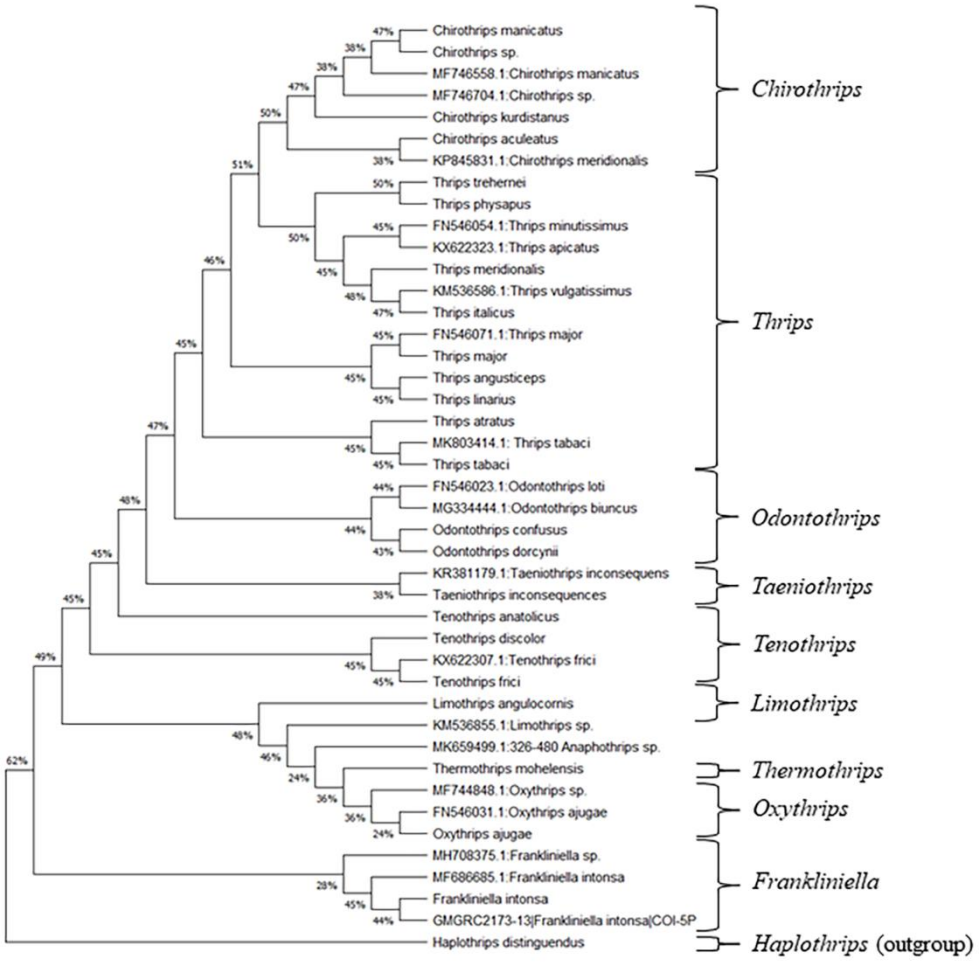

Figure 3
The UPGMA phylogenetic tree according to the COI gene region of Thripidae species studied with Mega X. *Haplothrips distinguendus* was used as outgroup. Sample sequences with numbers were taken from GenBank. (Overall mean distance: 0.24).

One of the best-known barcoding gen regions is COI to subserve as the core of a global bio identification system for insects (Hebert et. al., 2003b). The COI-based identification system provides the small number sampling of higher taxonomic categories and species-level assignments (Hebert et. al., 2003a). Secondly, the other cytochrome oxidase dataset as COIII also demonstrated acceptable loci for the diagnoses of the low-density of thrips according to Glover et. al. (2010). The dataset consisted of COI sequences supported the selected species morphological identification. Similarly, Fiala et. al. (2015) and Taddei et. al. (2021) stated that their thrips

COI sequences confirmed the species identifications based on morphological characters too. Our results agree with Kadirvel et. al. (2013a) reported the partial COI identification method successfully performed for at least 86% for the four major thrips species (*Thrips palmi* Karny, *Th. tabaci*, *Scirtothrips dorsalis* Hood and *F. occidentalis*). Marullo et. al. (2020) suggested that the COI barcoding system is useful to identify and classify thrips multiple species occurring in a crop system even though there were some misidentifications in their COI results, as they mentioned cause their technical problems such as ineffective primer selection.

The classical taxonomy has its own power, but DNA barcoding employing COI or 18S Ribosomal RNA has the added advantage for the target species to set up the suitable pest management strategies and quarantine processes (Rebijith et. al., 2013; Chakraborty et. al., 2019). A major current focus in integrated taxonomy is how to combine with morphology and molecular data successfully evidenced to identify the thrips species (Iftikhar et. al., 2016; Tyagi et. al., 2017). In this paper, the collected species of the 9-genera thrips from Turkey successfully identified via both classical and molecular taxonomy by both regions. Also, as Chakraborty et. al. (2019) we think that there has to be thorough taxonomic studies for more thrips species to diagnose with multiple molecular markers in the thrips systematics research. However, a complete and well-support reference library is an essential for molecular identification (Meyer and Paulay, 2005; Collins and Cruickshank, 2013). Apart from that, one of the key powers of the DNA barcoding method is the normalization of the locus used (Glover et. al., 2010).

The phylogenetic trees produced in the present study are assumed to be only a small step, and much work is needed at the species level. It has been realized that sampling in wider geographical areas aiming to collect the highest number of samples, possible, is required in Turkey in order to obtain barcoding data that would satisfactorily address the problems faced in conventional systematics of Thysanoptera.

The sequences obtained by Sanger sequencing from the COI and 18S Ribosomal RNA gene regions of the species studied were evaluated on the phylogenetic tree by UPGMA and NJ analysis. Considering the results of DNA barcode analysis with both markers, it may be assumed that the species-level distinction was possible for the COI gene region while the 18S Ribosomal RNA gene region did not yield the data required to discriminate satisfactorily at the species level.

Extensive mitochondrial sequencing information of all thrips species should be shared and included in phylogenetic studies since the phylogenetic trees made with DNA sequences have the potential to be misinterpreted Brunner et. al. (2002).

It can be concluded that these and similar studies, in which morphological and molecular diagnosis are made together. It is obvious that the rapid sequencing of a part of mitochondrial gene region by advanced molecular diagnostic studies will benefit science and agriculture. The use of COI gene regions, rather than the use of 18S Ribosomal RNA gene region seems more favorable for barcoding studies. The COI gene region, which gives the best diagnostic separation in the molecular method, should be completely extracted for all species, and should be enriched in the free and easy-to-access databases such as BOLD or GenBank.

4. Acknowledgements

This study is the part of doctoral thesis of İnci Şahin Negiş, titled “Morphological and molecular charac-terization of Thripidae (Thysanoptera) species in Karaman, Konya and Mersin” and supported by TUBITAK (Scientific and Technological Research Council of Turkey, 2211-C and 2214-A).

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