Experimental and Applied Acarology (2022) 87:195–205
https://doi.org/10.1007/s10493-022-00728-5

Molecular identification and phylogenetic analysis of spider mites (Prostigmata: Tetranychidae) of Turkey

Emre İnak[1] · Sultan Çobanoğlu[1] · Philippe Auger[2] · Alain Migeon[2]

Received: 5 April 2022 / Accepted: 23 June 2022 / Published online: 20 July 2022

Abstract
The family Tetranychidae includes many agriculturally important species known as spider mites. Their morphological identification is quite difficult due to the tiny size of their taxonomic characters and the requirement for high-level expertise. This may lead to pest misidentification and thus failure in pest management. DNA-based species identification seems to offer an alternative solution to overcome these issues. In the present study, two common molecular markers—*Cytochrome oxidase subunit I* (*COI*) and Internal transcribed spacer 2 (ITS2)—were used to identify 10 spider mite species from Turkey. Furthermore, genetic distances for several of them were assessed. *Panonychus ulmi* and *Bryobia kissophila* had the lowest (1.1%) and highest (4.5%) intra-specific genetic distances, respectively. In addition, integrative taxonomy allowed to identify *Eotetranychus quercicola* in Turkey as a new record. The sequences herein obtained will allow rapid species identification using molecular techniques and will contribute to resolve the phylogenetic history of spider mites.

Keywords Spider mites · Integrative taxonomy · Genetic distance · Tetranychidae · *Eotetranychus*

Introduction

Spider mites (Acari: Tetranychidae) are the most economically important mite family (Van Leeuwen et al. 2010). They can feed on various host plants and cause economic yield losses (Van Leeuwen et al. 2009). So far, more than 1300 tetranychid species have been recorded worldwide (Migeon and Dorkeld 2022). Misidentification of spider mites, as with pests in general, may cause failure in pest control.

Identification of tetranyhid mites is traditionally based on morphological characters (Seeman and Beard 2011), and there are several reasons that lead to species misidentification. High levels of experience and knowledge are required for species discrimination, particularly because of the minute size of the characters observed. Furthermore, among some

✉ Emre İnak
einak@ankara.edu.tr

[1] Department of Plant Protection, Faculty of Agriculture, Ankara University, Diskapi, 06110 Ankara, Turkey

[2] CBGP, INRAE, CIRAD, IRD, Montpellier SupAgro, Univ Montpellier, Montpellier, France

mite genera, only the males, which are less frequent in nature (Helle and Sabelis 1985), allow species discrimination. In addition, traditional morphological keys do not allow species discrimination of immature stages (Pritchard and Baker 1955).

DNA-based identification offers a solution to overcome these difficulties (Hebert et al. 2003; Ros and Breeuwer 2007). Several molecular markers have been used in species identification of mites so far (Navajas and Fenton 2000; Cruickshank 2002). The family Tetranychidae is among the first Acari groups used in molecular identification (Navajas et al. 1992). However, 30% of the sequences belonging to Tetranychidae in the public GenBank have been reported to be unreliable (de Mendonça et al. 2011). The internal transcribed spacer (ITS) nuclear gene region (ITS1-5.8S-ITS2) and mitochondrial *Cytochrome oxidase I* (*COI*) have been successfully used in the molecular identification of spider mites (Ben-David et al. 2007; Ros and Breeuwer 2007; Matsuda et al. 2014). Still, additional sequences are required to fully elucidate the intra- and inter-specific variations that allow more precise species identification.

Although about 30 spider mite species have been recorded in Turkey so far (Çobanoğlu et al. 2015; Altunç and Akyazı 2020; Migeon and Dorkeld 2022), *Tetranychus urticae* Koch is the only species having *COI* sequences (İnak et al. 2019), and there are no ITS sequences obtained from spider mites in Turkey. In this study, we molecularly characterize ten tetranychid species using two genes collected from various hosts in Turkey. In addition, a new record belonging to the genus *Eotetranychus* has been reported using integrative taxonomy.

Materials and methods

Collection and identification of tetranychid mites

Tetranychid mites were collected from various regions of Turkey during 2019–21. Only plants that showed spider mite damage were sampled. Mites were transferred to 70 and 96% alcohol for morphological and molecular identification, respectively. Locations, hosts, collection dates and accession numbers are presented in Table 1.

Hoyer's medium was used for the permanent slides and male individuals have been positioned laterally to examine the aedeagus. The permanent slides are deposited at Ankara University, Department of Plant Protection, and at INRAE (Montpellier, France) in case of future verification.

Morphological identifications were performed mainly using the following publications: Pritchard and Baker (1955), Jeppson et al. (1975), Hatzinikolis and Emmanouel (1991), Zhang (2003), Auger et al. (2003) and Auger and Migeon (2014). In addition, original descriptions of *Eotetranychus* species were used when necessary.

Genomic DNA extraction

The mites kept in 96% alcohol were placed on filter paper allowing for the evaporation of alcohol. Genomic DNA was extracted from individual adult females using a Qiagen DNaeasy Blood and Tissue Kit following the manufacturer's instructions. In the final step, 50 µl

Table 1 Collection dates and locations, host plants and accession numbers of spider mite species obtained in this study

Species	Code	Location	Host	Collection date	Accession number	
					COI	ITS
Tetranychus urticae	KC	Konya/Çumra	*Phaseolus vulgaris*	10.09.2019	MW535081	MW534221
	KA	Konya/Altınekin	*Phaseolus vulgaris*	15.09.2019	MW535080	MW534222
Tetranychus turkestani	NalT1, NalT2, NalT3	Ankara/Nallıhan/ Akçabayır	Mixed	21.05.2020	MW535082, MW535083, MW535084	MW534220, MW534218, MW534219
	NkusT2, NkusT4	Ankara/Nallıhan	Mixed	21.05.2020	MW535085, MW535086	MW534217, MW534216
Amphitetranychus viennensis	AEMT1-2–3-4–5	Ankara/Çankaya/Eymir gölü	*Prunus domestica*	01.10.2019	MW535087, MW535088, MW535089, MW535090, MW535091	MW534210, MW534215, MW534214, MW534206, MW534211
	YV2, YV5	Yozgat/Aydıncık/Kazankaya	*Prunus cerasus*	08.09.2019	MW535097, MW535096	MW534205, MW534204
	YK3	Yozgat/Aydıncık/Kazankaya	*Prunus avium*	08.09.2019	MW535098	MW534207
	CK5, CK8	Ankara/Çamlıdere	*Prunus domestica*	07.07.2019	MW535094, MW535093	MW534212, MW534213
	Bas4, Bas10	Ankara/Ayaş/Başbereket	*Prunus domestica*	17.05.2019	MW535092, MW535095	MW534209, MW534208
Eurytetranychus buxi	BK	Ankara/Keçiören	*Buxus sempervirens*	12.09.2019	MW535073	MW534201
	BIS4	Bilecik/Bilecik Şeyh Edebali University	*Buxus sempervirens*	04.10.2019	MW535072	MW534200
Eutetranychus orientalis	PST2	Antalya/Kumluca	*Citrus sinensis*	10.08.2019	MW535071	MW534202
	A1	Adana/Seyhan	*Citrus sinensis*	7.09.2019	MW535070	MW534203
Eotetranychus populi	Ihl3, Ihl5	Ankara/Çankaya	*Tilia cordata*	3.07.2020	MW535075, MW535076	MW530357, MW530358
Eotetranychus quercicola	Me1, Me2,Me3	Ankara/Keçiören	*Quercus* sp.	2.07.2020	MW535077, MW535078, MW535079	MW534197, MW534198, MW534199
Panonychus ulmi	AK1	Antalya/Kaş/Gömbe	*Malus domestica*	30.06.2020	MW535074	MW530359
Bryobia rubrioculus	B1,2,3	Ankara/Ayaş/Başbereket	*Malus domestica*	17.05.2019	MW535102, MW535103, MW535104	MW530360, MW530362, MW530361
Bryobia kissophila	CBr1,2,3	Ankara/Çankaya	*Hedera helix*	20.05.2020	MW535099, MW535100, MW535101	MW530363, MW530364, MW530365

of elution buffer was used for each sample (İnak et al., 2020). DNA extracts were stored at −20 °C until used in PCR reactions.

Amplification of target gene/gene regions

Primers used for amplifying the target genes (ITS and *COI*) are given in Table 2. The PCR reaction was performed in a total volume of 30 µl, containing 5 µl of mite DNA, 0.5 µl of both forward and reverse primers, 18 µl of ultrapure nuclease-free water and 6 µl of FIRE-Pol Master Mix (Solis Biodyne, Estonia).

DNA amplification was performed with a thermal cycler (BioRad T100) under the following conditions: 4 min at 94 °C, 40 cycles of 60 s at 92 °C, 60 s at 48–51 °C and 90 s at 72 °C, followed by a final extension of 5 min at 72 °C. Electrophoresis was carried out on a 1.5% agarose gel in 0.5 × TAE buffer for 35 min at 100 V to verify the success of the PCR.

The post-PCR products were purified using the HighPrep PCR clean-up system (MagBio Genomics, Gaithersburg, MD, USA) and sequencing was performed at the company BM (Ankara, Turkey).

Phylogenetic analysis

The obtained sequencing chromatographs were checked for errors based on visual inspection using BioEdit v.7.0.5 (Hall 1999). Then, all sequences herein obtained (MW535070-MW535104 for *COI*, MW534197-MW534222 for ITS) and retrieved from the public GenBank were aligned using MAFFT v.7 with 'Auto' strategy (Katoh et al. 2019) and refined manually. Maximum likelihood (ML) phylogenetic trees were built with MEGA X using the GTR + G + I for COI and T92 + G + I model for ITS (identified to be the best-fit model in MEGA X) (Tamura 1992) with 1000 bootstraps.

Intra- and interspecific genetic distances were calculated using all available sequences in the public GenBank (see Table S1 for the accession numbers of the used sequences) with the K2Parameter model in Mega X via selecting the target sequences as groups and computing the distances between/within them (Kumar et al. 2018).

Table 2 Primers and melting temperatures (TM) used in amplification of the target gene/gene regions

Target gene/gene region	Primers	Sequences	Melting temperature (°C)	Reference
ITS	TuITS_F	AGAGGAAGTAAAAGTCGTAACAAG	50	Navajas et al. 1999
	TuITS_R	ATATGCTTAAATTCAGGGGG		
	ITS_F	GTCACATCTGTCTGAGAGTTGAGA	51	Ben-David et al. 2007
	ITS_R	GTARCCTCACCTRMTCTGAGATC		
COI	COI_F1	TGATTTTTTGGTCACCCAGAAG	48	Ros et al. 2008
	COI_F3	WGTHTTAGCAGGAGCAATTACWAT		
	COI_F4	GGAGGATTTGGAAATTGATTAGTTCC		
	COI_R1	TACAGCTCCTATAGATAAAAC		
	COI_R2	AAWCCTCTAAAAATRGCRAATACR		

Results and discussion

Although most of the species obtained in the present study have been reported previously in Turkey, no sequence data belonging to these species (except *COI* sequences of *T. urticae*) have been available so far. Considering the importance of the determination of genetic variation within certain species from different geographical areas, there was a gap in the literature for the sequences of Turkish spider mites.

A total of 70 sequences (*COI* + ITS, 35 sequences for each) belonging to ten tetranychid species have been obtained: *Bryobia kissophila* van Eyndhoven, *Bryobia rubrioculus* (Scheuten), *Eutetranychus orientalis* (Klein), *Eurytetranychus buxi* (Garman), *Amphitetranychus viennensis* (Zacher), *Eotetranychus populi* (Koch), *Eotetranychus quercicola* Auger & Migeon, *Panonychus ulmi* (Koch), *T. urticae* and *Tetranychus turkestani* Ugarov & Nikolski. Morphological identification was further supported by sequence comparison (COI max distance = 1.74%) and thus the integrative approach allowed to identify a new record of *E. quercicola* for the mite fauna of Turkey—this species was only known from South France, collected on *Quercus pubescens* (see Auger and Migeon 2014 for the detailed description).

The phylogenetic tree based on *COI* sequences is presented in Fig. 1. The tree shows a good clustering pattern at the genus level, although it was not supported by high bootstrap values. The genus *Eotetranychus* shows a polyphyletic evolutionary history in line with Matsuda et al. (2014; 2018). The genera *Eutetranychus* and *Eurytetranychus*, which belong to the tribe Eurytetranychini, clustered together, in line with Matsuda et al. (2014), supporting their closely related history. However, there is only a limited number of *COI* sequences belonging to spider mites in the public GenBank; therefore, more sequences from various species are needed to analyse the suitability of *COI* as a molecular marker for genus-level discrimination. At the species-level, the *COI* gene seems to allow species discrimination with some exceptions (i.e., closely related species such as *T. urticae* and *T. turkestani*, only 1.9% genetic difference between Turkish specimens), in line with Hinomoto et al. (2007) and Ros and Breeuwer (2007). Similarly, Matsuda et al. (2013) separated 13 tetranychid species from Japan using *COI* sequences successfully, on the other hand, only 10 out of these 13 species have been identified using the ITS region.

The intra- and inter-specific genetic variation for three tetranychid species that have more than 25 sequences available in GenBank were assessed (Table 3). The gap between intra- and inter-specific genetic distance (also known as barcoding gap) indicates the usefulness of *COI* sequences in species identification of analysed spider mites. *Panonychus ulmi* and *B. kissophila* had the lowest (1.09%) and highest (4.48%) intra-specific genetic distance, respectively. Besides commonly distributed species herein analysed, assessment of barcoding gaps should be performed for cryptic and closely related spider mite species where species delimitation is of significant importance in further studies. Rather than using a single or few methods in species delimitation, a wide range of analyses should be applied and all available data should be integrated to establish a standard barcoding gap (Carstens et al., 2013).

Although Navajas et al. (1999) and Choi et al. (2018) reported higher genetic distances within *A. viennensis* when all sequences available in GenBank were analysed (up to 4.1 and 6.9%, respectively), the mean genetic distance was 2.63%. Still, *COI* has been determined to be a suitable marker for identification of *A. viennensis* in the present study, in line with Arabuli et al. (2019) that discriminated all three species belonging to the genus *Amphitetranychus* using *COI* sequences.

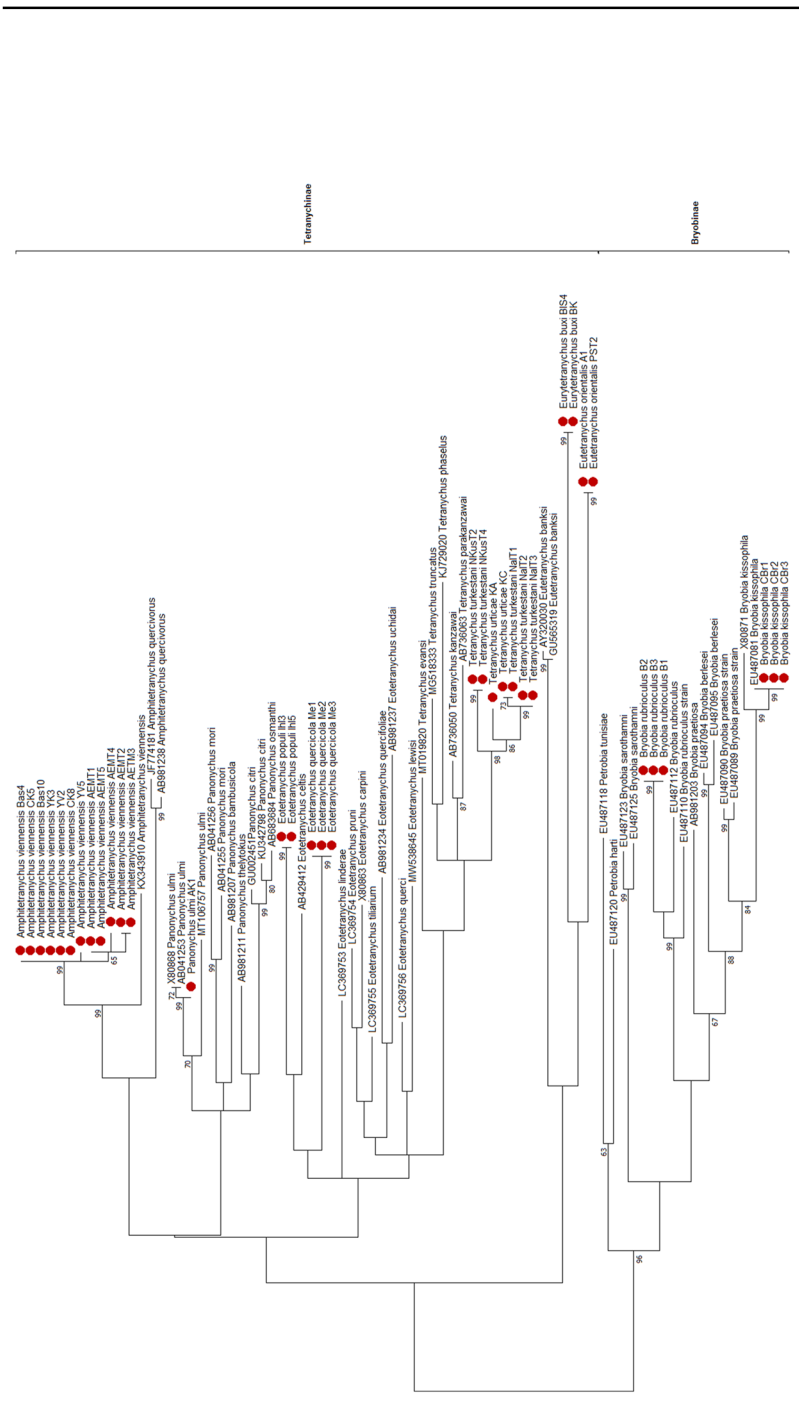

Fig. 1 Phylogenetic tree of obtained tetranychid species based on *COI* sequences. The red dots indicate the sequences obtained in the present study. Only the bootstrap values higher than 60% are shown

Table 3 Mean (±SE) intra- and interspecific genetic distances for four tetranychid species based on *COI* sequences

Species	No. specimens	Intra-specific distance	Inter-specific distance
Amphitetranychus viennensis	45	0.026 ± 0.005	0.106 ± 0.016
Panonychus ulmi	105	0.010 ± 0.002	0.097 ± 0.016
Bryobia kissophila	25	0.044 ± 0.007	0.122 ± 0.013

ITS2 evolves very quickly, which makes it a suitable marker in phylogenetic analysis (Yao et al. 2010). Although alignment of ITS2 sequences might sometimes be difficult due to its high sequence divergence, its secondary structure has been conserved considerably. It therefore allows identification at species-level (Schultz and Wolf 2009; Koetschan et al. 2010). In addition, Navajas et al. (1998) reported that ITS evolves 2.5 × faster than *COI* in spider mites, indicating the presence of a high level of differentiation within and between species.

The ITS2-based phylogenetic tree is presented in Fig. 2. Contrary to the *COI*-based tree, the genera *Amphitetranychus* and *Tetranychus* were seperated with high bootstrap values (91%) in the tree constructed using ITS2 sequences. Both trees showed the monophyly of the genera *Tetranychus*, *Amphitetranychus* and *Bryobia*, although the genus *Tetranychus* has been suggested to be polyphyletic (Matsuda et al. 2014, 2018). However, the number of sequences analysed in the present study is not sufficient to show a high-resolution phylogenetic relationship. The dataset and markers analysed (even different partial fragments of the same gene/gene regions) could also lead to such incongruence in the results. In addition, concatenated trees using multiple genes or phylogenomics, if possible, may allow overcoming this issue (Gadagkar et al. 2005; Delsuc et al. 2005). Still, misidentified and contaminated DNA sequences should also be taken into account before drawing a conclusion (de Mendonça et al. 2011).

Tetranychus urticae and *T. turkestani* were separated according to nucleotide substitution at certain positions in ITS as previously described (Navajas and Boursot 2003; de Mendonça et al. 2011). These species did not cluster together in the ITS-based phylogenetic tree (contrary to the *COI*-based tree), which might allow the discrimination of even such closely related species using phylogenetic trees, in line with Navajas and Boursot (2003) and Ben-David et al. (2007).

The discrepancy between the phylogenetic trees based on nuclear and mitochondrial markers (although showing similar higher-level taxonomic composition) might be associated with insufficient data, homoplasy, incomplete lineage sorting and different hybridization events.

Conclusion

Accurate and early identification of spider mites is the initial step in pest management. Although DNA-based species identification is gaining more and more importance, the lack of reliable reference sequences and knowledge on genetic variation within and between genera/species often limits the use of this technique. In this study, ten tetranychid species collected in Turkey were molecularly characterized using the *COI* and ITS sequences. Whereas almost all the sequences obtained represent the first sequences from Turkey, some of them were submitted to the public GenBank for the first time. In addition, DNA-based

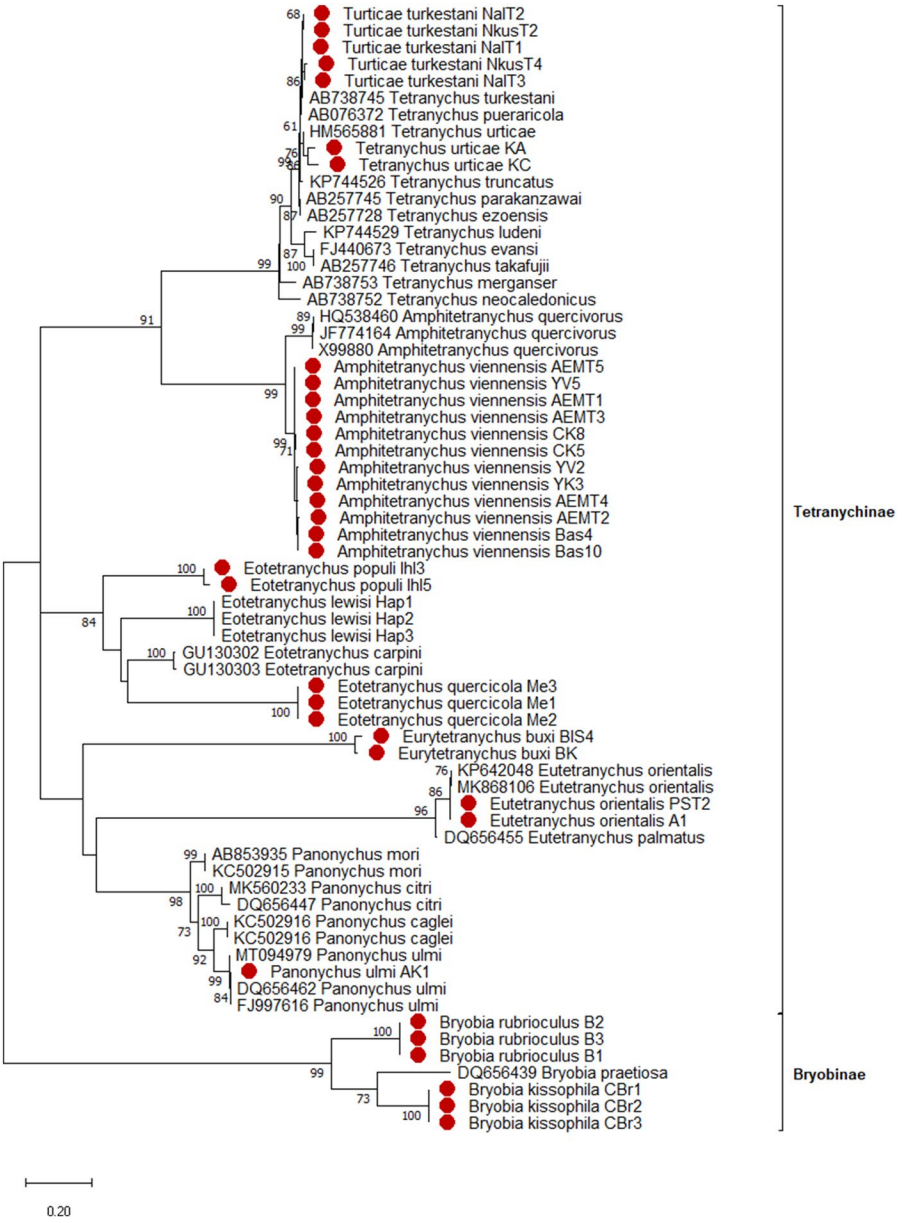

Fig. 2 Phylogenetic tree of obtained tetranychid species based on ITS sequences. The red dots indicate the sequences obtained in the present study. Only the bootstrap values higher than 60% are shown

species identification allowed us to provide a new spider mite record for Turkey. In the future, together with increasing reference sequences, it might be possible to perform faunistic studies using molecular data for mite groups as indiviual samples or pools of mites/samples using next generation sequencing. In this context, the establishment of a reference mite database based on integrative taxonomy that provides more reliable results

would substantially contribute to all aspects of acarological studies including ecology, evolution, biological control and pest management. Finally, the number of species and individuals used in genetic distance assessments varied from one specimen to another, which might cause misconceptions; therefore, more sequences are required to obtain more accurate results.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10493-022-00728-5.

Acknowledgements The authors would like to thank to everyone who provides plant samples. This study is a part of the PhD thesis of first author.

Author contributions Eİ and SÇ: conceived the ideas. Eİ: performed the experiments. All the authors analysed the data. Eİ, PA and AM: wrote the main manuscript text. All authors read and approved the final manuscript.

Funding This research was supported by Ankara University Scientific Research Projects Coordination Unit. Project Number 19L0447009, 2021.

Data availability All data and materials are available for publication.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to disclose.

Ethical approval Not applicable.

Consent to participate All authors consented to participate.

Consent for publication All authors read and approved the fnal manuscript.

References

Altunç YE, Akyazı R (2020) Two New Records for Spider Mite Fauna of Turkey, *Tetranychus kanzawai* Kishida and *Eotetranychus rubiphilus* Reck (Trombidiformes: Tetranychidae). Turkish J Agric-Food Sci Technol. https://doi.org/10.24925/turjaf.v8i8.1598-1602.2387

Arabuli T, Negm MW, Matsuda T, Kitashima Y, Abramishvili T, Akimov IA, Zhovnerchuk OV, Popov SY, Gotoh T (2019) Morphological identification of *Amphitetranychus* species (Acari: Tetranychidae) with crossbreeding, esterase zymograms and DNA barcode data. PLoS ONE 14(9):e0221951. https://doi.org/10.1371/journal.pone.0221951

Auger P, Migeon A (2014) Three new species of Tetranychidae (Acari, Prostigmata) from the French Alps (South-Eastern France). Acarologia 54(1):15–37. https://doi.org/10.1051/acarologia/20142111

Auger P, Migeon A, Flechtmann CHW (2003) A new species of *Eotetranychus* from France (Acari, Prostigmata: Tetranychidae). Zootaxa 206(1):1–7

Ben-David T, Melamed S, Gerson U, Morin S (2007) ITS2 sequences as barcodes for identifying and analyzing spider mites (Acari: Tetranychidae). Exp Appl Acarol 41(3):169–181. https://doi.org/10.1007/s10493-007-9058-1

Carstens BC, Pelletier TA, Reid NM, Satler JD (2013) How to fail at species delimitation. Mol Ecol 22(17):4369–4383. https://doi.org/10.1111/mec.12413

Choi CW, Shim JK, Jung DO, Lee KY (2018) Genetic variation of the hawthorn spider mite *Amphitetranychus viennensis* (Acari: Tetranychidae) in Korea. Entomol Res 48(3):165–173. https://doi.org/10.1111/1748-5967.12255

Çobanoğlu S, Ueckermann EA, Kumral NA (2015) A new *Tetranychus* Dufour (Acari: Tetranychidae) associated with Solanaceae from Turkey. Turk J Zool 39(4):565–570. https://doi.org/10.3906/zoo-1406-17

Cruickshank RH (2002) Molecular markers for the phylogenetics of mites and ticks. Syst Appl Acarol 7(1):3–14. https://doi.org/10.11158/saa.7.1.1

de Mendonça RS, Navia D, Diniz IR, Auger P, Navajas M (2011) A critical review on some closely related species of *Tetranychus* sensu stricto (Acari: Tetranychidae) in the public DNA sequences databases. Exp Appl Acarol 55(1):1–23. https://doi.org/10.1007/s10493-011-9453-5

Delsuc F, Brinkmann H, Philippe H (2005) Phylogenomics and the reconstruction of the tree of life. Nat Rev Genet 6(5):361–375. https://doi.org/10.1038/nrg1603

Gadagkar SR, Rosenberg MS, Kumar S (2005) Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. J Exp Zool 304(1):64–74. https://doi.org/10.1002/jez.b.21026

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hatzinikolis EN, Emmanouel NG (1991) A Revision of the Genus *Bryobia* in Greece (Acari: Tetranychidae). Entomol Hell. 9:21–34. https://doi.org/10.12681/eh.13989

Hebert PD, Ratnasingham S, De Waard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc R Soc b: Biol Sci 270(suppl_1):S96–S99. https://doi.org/10.1098/rsbl.2003.0025

Helle W, Sabelis MW (1985) Spider mites: their biology, natural enemies and control. Elsevier, Amsterdam

Hinomoto N, Tran DP, Pham AT, Le Ngoc TB, Tajima R, Ohashi K, Osakabe M, Takafuji A (2007) Identification of spider mites (Acari: Tetranychidae) by DNA sequences: a case study in Northern Vietnam. Int J Acarology 33(1):53–60. https://doi.org/10.1080/01647950708684501

İnak E, Alpkent YN, Çobanoğlu S, Dermauw W, Van Leeuwen T (2019) Resistance incidence and presence of resistance mutations in populations of *Tetranychus urticae* from vegetable crops in Turkey. Exp Appl Acarol 78(3):343–360. https://doi.org/10.1007/s10493-019-00398-w

İnak E, Çobanoğlu S, Sade E, Tixier MS (2020) Molecular characterization of phytoseiid mites in Turkey based on the internal transcribed spacer (ITS) region, with a new record for the country. Exp Appl Acarol 81:201–213. https://doi.org/10.1007/s10493-020-00504-3

Jeppson LR, Keifer HH, Baker EW (1975) Mites injurious to economic plants. Univ of California Press, California

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinformatics 20(4):1160–1166. https://doi.org/10.1093/bib/bbx108

Koetschan C, Foerster F, Keller A, Schleicher T, Ruderisch B, Schwarz R, Müller T, Wolf M, Schultz J (2010) The ITS2 Database III—sequences and structures for phylogeny. Nucleic Acids Res 38(suppl_1):D275–D279. https://doi.org/10.1093/nar/gkp966

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096

Matsuda T, Fukumoto C, Hinomoto N, Gotoh T (2013) DNA-based identification of spider mites: molecular evidence for cryptic species of the genus *Tetranychus* (Acari: Tetranychidae). J Econ Entomol 106(1):463–472. https://doi.org/10.1603/EC12328

Matsuda T, Morishita M, Hinomoto N, Gotoh T (2014) Phylogenetic analysis of the spider mite subfamily Tetranychinae (Acari: Tetranychidae) based on the mitochondrial COI gene and the 18S and the 5′ end of the 28S rRNA genes indicates that several genera are polyphyletic. PLoS ONE 9(10):e108672. https://doi.org/10.1371/journal.pone.0108672

Matsuda T, Kozaki T, Ishii K, Gotoh T (2018) Phylogeny of the spider mite sub-family Tetranychinae (Acari: Tetranychidae) inferred from RNA-Seq data. PLoS ONE 13(9):e0203136. https://doi.org/10.1371/journal.pone.0203136

Migeon A, Dorkeld F (2022) Spider Mites Web: a comprehensive database for the Tetranychidae. Available from . Accessed 21 February 2022 http://www1.montpellier.inra.fr/CBGP/spmweb

Navajas M, Boursot P (2003) Nuclear ribosomal DNA monophyly versus mitochondrial DNA polyphyly in two closely related mite species: the influence of life history and molecular drive. Proc. Royal Soc B 270(suppl_1):S124–S127. https://doi.org/10.1098/rsbl.2003.0034

Navajas M, Fenton B (2000) The application of molecular markers in the study of diversity in acarology: a review. Exp Appl Acarol 24(10):751–774. https://doi.org/10.1023/A:1006497906793

Navajas M, Cotton D, Kreiter S, Gutierrez J (1992) Molecular approach in spider mites (Acari: Tetranychidae): preliminary data on ribosomal DNA sequences. Exp Appl Acarol 15(4):211–218. https://doi.org/10.1007/BF01246563

Navajas M, Lagnel J, Gutierrez J, Boursot P (1998) Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. Heredity 80(6):742–752. https://doi.org/10.1046/j.1365-2540.1998.00349.x

Navajas M, Gutierrez J, Lagnel J, Fauvel G, Gotoh T (1999) DNA sequences and cross-breeding experiments in the hawthorn spider mite *Amphitetranychus viennensis* reveal high genetic differentiation between Japanese and French populations. Entomol Exp Appl 90(2):113–122. https://doi.org/10.1046/j.1570-7458.1999.00429.x

Pritchard AE, Baker EW (1955) A revision of the spider mite family Tetranychidae. Memoirs Series, San Francisco, Pacific Coast Entomological Society, California

Ros VI, Breeuwer JA (2007) Spider mite (Acari: Tetranychidae) mitochondrial COI phylogeny reviewed: host plant relationships, phylogeography, reproductive parasites and barcoding. Exp Appl Acarol 42(4):239–262. https://doi.org/10.1007/s10493-007-9092-z

Ros VI, Breeuwer JA, Menken SB (2008) Origins of asexuality in Bryobiamites (Acari: Tetranychidae). BMC Evol Biol 8:153. https://doi.org/10.1186/1471-2148-8-153

Schultz J, Wolf M (2009) ITS2 sequence–structure analysis in phylogenetics: a how-to manual for molecular systematics. Mol Phylogenet Evol 52(2):520–523. https://doi.org/10.1016/j.ympev.2009.01.008

Seeman OD, Beard JJ (2011) Identification of exotic pest and Australian native and naturalised species of *Tetranychus* (Acari: Tetranychidae). Zootaxa 2961(1):1–72. https://doi.org/10.11646/zootaxa.2961.1.1

Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Mol Biol Evol 9(4):678–687. https://doi.org/10.1093/oxfordjournals.molbev.a040752

Van Leeuwen T, Vontas J, Tsagkarakou A, Tirry L (2009) Mechanisms of acaricide resistance in the two-spotted spider mite *Tetranychus urticae*. Ishaaya I Horowitz AR Biorational control of arthropod pests. Springer, Dordrecht, pp 347–393

Van Leeuwen T, Vontas J, Tsagkarakou A, Dermauw W, Tirry L (2010) Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Insect Biochem Mol 40(8):563–572. https://doi.org/10.1016/j.ibmb.2010.05.008

Yao H, Song J, Liu C, Luo K, Han J, Li Y, Pang X, Xu H, Zhu Y, Xiao P, Chen S (2010) Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS ONE 5(10):e13102. https://doi.org/10.1371/journal.pone.0013102

Zhang ZQ (2003) Mites of greenhouses: identification, biology and control. Cabi, UK