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***Orijinal araştırma* (*Original article*)**

Re-discovery and identification of *Iphiseius degenerans* (Acari: Phytoseiidae) in Turkey, based on morphological and molecular data[1]

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Türkiye'de *Iphiseius degenerans* (Acari: Phytoseiidae)'ın yeniden saptanması ve moleküler-morfolojik verilere bağlı olarak tanımlanması

Abstract: The monotypic genus *Iphiseius* is represented by only *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae). This species, which is one of the most common predatory mites in citrus orchards in Mediterranean countries, is an important biological control agent of various pests that include thrips, whiteflies and spider mites. This species was included in a brief report of the Turkish fauna some 50 years ago. However, there was no morphological information, illustrations or collection details of the examined specimens, except for its host plant, sour lemon, and an unknown locality in Mersin Province, Turkey. Since that time, extensive surveys conducted in citrus plantations in both Mersin and Adana Provinces have not confirmed its presence. However, in 2008, 2011 and 2013, a natural population of *I. degenerans* was encountered in Anıtlı town, near the border with Antalya Province, where it was associated with thrips on a non-cultivated host, *Hedera helix* L. (Araliaceae). In this study, we re-describe *I. degenerans*, based on both female and male specimens, and provide all morphological details. The DNAs of the specimens were successfully isolated and amplified using an internal transcribed spacer (ITS) gene marker by polymerase chain reaction (PCR). A phylogenetic tree was constructed using the DNA sequence of the amplified region, as well as other sequences deposited in the National Center for Biotechnology Information (NCBI). The phylogenetic tree and genetic divergence were constructed and estimated, respectively, using the Jukes and Cantor models, respectively. There were no morphological differences in comparison to other populations of *I. degenerans*. This result was confirmed by the molecular study as no genetic divergence with other populations was found. The results of this study will be useful for further systematic studies on theTurkish Phytoseiidae, and would also help non-expert, phytoseiid taxonomists to correctly identify *I. degenerans.*

[1]This study was presented at the IOBC Meeting of the Working Group on "*Integrated Control of Plant-Feeding Mites*" that was held from 4-7 September, 2017 in Chania, Greece, and appears in the abstract book of that meeting.

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Öz: *Iphiseius* cinsi sadece *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae) tarafından temsil edilen monotipik bir cinstir. Bu tür Akdeniz ülkeleri turunçgil bahçelerinde bulunan en yaygın avcı akarlardan biri olup, kırmızıörümcek, beyazsinek ve thrips gibi birçok zararlının biyolojik mücadelesinde kullanılan önemli bir doğal düşmandır. Bu türün Türkiye faunası için varlığı yaklaşık 50 yıl önce Mersin ilinde limon ağaçlarında saptanmış morfolojik özelliklere ve çizimlere yer verilmeden kısaca bildirilmiştir. Ancak daha sonra Adana ve Mersin illeri turunçgil bahçelerinde yapılan kapsamlı sörvey çalışmalarında bu türe rastlanılmamıştır. *Iphiseius degenerans*'ın doğal populasyonu Mersin ili Anıtlı köyünde thrips ile bulaşık *Hedara helix* (Araliaceae) bitkileri üzerinde 2008, 2011 ve 2013 yıllarında saptanmıştır. Bu çalışmada, *I. degenerans'*ın tanısı erkek ve dişi bireyler üzerinden yeniden yapılmış ve bütün morfolojik özellikleri modern tanımlamalara göre verilmiştir. Avcı akarın DNA'sı ITS gen bölgesine ait primer kullanılarak PZR (Polimeraz Zincir Reaksiyonu) ile elde edilmiş ve çoğaltılmıştır. Filogenetik ağaç, çalışmada elde edilen sekanslar ve NCBI gen bankasında depolanan sekanslar kullanılarak oluşturulmuştur. Filogenetik ağaç ve genetik uzaklık Jukes & Cantor modeline göre oluşturulmuş ve hesaplanmıştır. Sonuçlara göre *I. degenerans*'n Türkiye populasyonu ile diğer populasyonlar arasında herhangi bir morfolojik farklılık gözlemlenmemiştir. Morfolojik olarak saptanan bu sonuç moleküler yöntemler ile desteklenmiş ve yapılan filogenetik analizlerde tüm *I. degenerans* türleri aynı grup içerisinde yer almıştır. Bu çalışmanın sonuçları Türkiye'de klasik yöntemler ile yapılan phytoseiid teşhislerinin desteklenmesi açısından fayda sağlayacak, ayrıca uzman olmayan araştırıcıların teşhis yapabilmelerine yardımcı olacaktır.

Anahtar kelimeler: *Iphiseius degenerans*, ITS bölgesi, genetik uzaklık, filogenetik ağaç, Türkiye

Introduction

Predatory mites belonging to the family Phytoseiidae (Acari: Mesostigmata) are widely used as biological control agents in many agricultural systems, especially in protected crops (Chant & McMurtry 2007; Papadoulis et al. 2009; McMurtry et al. 2013). Proper identification of species is the first and the most important step for biological control and integrated pest management (IPM) programs. Many new phytoseiid descriptions are still mainly based on the morphological characters that include dorsal setal length, shape of calyx of spermatheca, length of peritreme and number of dorsal solenostomes, with the application of molecular tools limited to only a few recent studies (Chant & McMurtry 2007; Tsolakis et al. 2012; Santos & Tixier 2017). However, observation of the important morphological characters on slide mounted specimens is sometimes difficult and thus may lead to misidentification because the phytoseiids are less than 500 micrometers (μm) in body length. Therefore, for the proper identification of species with the classical methods, an expert is always needed. However, contrary to the classical methods, molecular tools can be used for final confirmation.

The genus *Iphiseius* is a monotypic genus represented by only *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae). It is one of the most common predatory mites in citrus orchards in Mediterranean countries, being an important bio-control

agent of various pests such as thrips, whiteflies and spider mites (Fantinou et al. 2012; Döker et al. 2015).This species was reported for the first time for the Turkish fauna by Düzgüneş (1963), without morphological information, illustrations or the deposition of any examined materials. According to the brief report of Düzgüneş (1963), the natural population of *I. degenerans* was collected from lemon trees in Mersin Province by staff of the Adana Plant Protection Research Institute. However, all further attempts to re-collect it from this host and locality were unsuccessful (Şekeroğlu 1984; Yıldız 1998; Kasap 2001; Kazak et al. 2016). This may be due to the extensive usage of pesticides, including acaricides, in citrus orchards (Döker & Kazak 2012). The native population of *I. degenerans* used in the present study was encountered in the town Anıtlı in Mersin Province, Turkey on a non-cultivated host. In this study, a detailed morphological re-description of a Turkish population of *I. degenerans*, based on female and male specimens, is provided. In addition, its molecular characterization using an ITS gene region primer is also given.

Materials and methods

Predatory mite sources and morphological investigations

Plant samples were collected in Anıtlı (Mersin Province, Turkey) in the years 2008, 2011 and 2013. The samples were wrapped in paper towels and placed in plastic bags and then in a cool box. The samples were examined under a stereomicroscope and the collected mites were transferred to detached bean leaves that were placed on water saturated cotton wool in plastic cups. Sufficient amounts of cattail (*Typha latifolia* L. (Typhaceae)) pollen was dusted on the bean leaves as an alternative food source for the predatory mites to avoid contamination by the DNA of ingested prey. The predators were kept in rearing units at 25±2 °C, 70±5% RH and 16 hours photoperiod for 10 days on that diet. After that period, all surviving individuals were removed from the rearing units and the eggs (F_1 generation) were monitored until they reached the adult stage. The mites were then transferred to 98% alcohol and lactic acid for molecular and morphological investigations, respectively. For clearing, the mites were kept in lactic acid on a hotplate at 50°C for 24 hours. Permanent microscope slides were prepared from the females of the F_1 generation using Hoyer's medium and were identified as *I. degenerans* (Chant & McMurtry 2005; Moraes et al. 2007; Papadoulis et al. 2009; Döker et al. 2014a). An Olympus® CX41 microscope with the Olympus® U-DA drawing attachment, camera Lucida, was used for the illustrations. The taxonomic system employed was based on that proposed by Chant & McMurtry (2007). The setal nomenclature used followed Lindquist & Evans (1965), as adapted by Rowell et al. (1978); the organotaxy nomenclature used followed Athias-Henriot (1975; 1977), Evans & Till (1979) and Evans (1963) for the ventral pores and leg chaetotaxy; and followed Wainstein (1973) for the spermatheca, as proposed by Papadoulis et al. (2009). The dorsal and ventral setal pattern used was that of Chant & Yoshida-Shaul (1989; 1991; 1992). All measurements are given in μm and presented as the mean, followed by the range

in parentheses. Permanent voucher slides were deposited in the mite collection of the Acarology Laboratory, Cukurova University, Adana, Turkey.

Molecular Investigations

DNA of *I. degenerans* was isolated from the female specimens that had been fed on cattail pollen for 10 days. DNA was extracted from three adult females using a PureLink® genomic DNA kit (QIAGEN Inc., Dusseldorf, Germany), PCR products were generated from a nuclear gene ITS region. The extracted DNAs were amplified by using the primer pairs, 5'AGAGGAAGTAAAAGTCGTAACAAG3', and 5'ATATGCTTAAATTCAGGGGG3', and for the end of 28 rDNA (Navajas et al. 1999). Electrophoresis was carried out in a 1.5% agarose gel in 0.5 X TBE buffer for 60 min at 60 volts. The positive DNA samples were sequenced by MEDSANTEK (İstanbul, Turkey).

Analyses of DNA sequences

Contigs were constructed by alignment of the forward and reverse sequences by using the BioEdit computer program (Hall 1999). The genetic divergence between the phytoseiid mites were estimated by using the Jukes-Cantor model (Jukes & Cantor1969). The phylogenetic tree was constructed using the maximum likelihood method (ML), based on the Jukes-Cantor model with 1000 bootstraps, in Mega 7 (Kumar et al. 2016). The sequences of two *Euseius* species, *E. stipulatus* (Accession numbers: KY751690, KP642043) and *E. scutalis* (KP642044), were included in the phylogenetic analyses to improve the precision of the tree because *Euseius* and *Iphiseius* are two closely related genera in the same tribe (Euseiini) and subtribe (Euseiina) (Chant & McMurtry 2007). The sequence of another phytoseiid mite, *Amblydromalus limonicus* (Garman & McGregor) (HM189291), belonging to same tribe but in a different subtribe (Typhlodromalina), was included as an outgroup.

Results

Morphological investigations

Re-description of *Iphiseius degenerans* (Berlese)
Seius degenerans Berlese, 1889: 9.

Female (Figures 1–5) (n=10)

Dorsum (Figure 1). Dorsal setal pattern 10A:9B (r3 and R1 off shield). Dorsal shield oval without waist, strongly sclerotized and smooth, living individuals' dark and brownish in color. The dorsal shield bearing eight pairs of small and rounded solenostomes. Muscle-marks (sigilla) visible mostly on podosoma, length of dorsal shield (j1–J5) 383 (378–388), width (distance between bases of s4) 263 (260–265), width (distance between bases of S2) 274 (270–278). Dorsal setae minute except for j1 and Z5, which are slightly longer. All dorsal setae smooth. Measurements of dorsal setae as follows: j1 24 (20–28), j3, j4, j5, j6, J2, J5, z2, z4, z5, Z1, Z4, 4 (3–5), Z5 11 (10–13), s4, S2, S4, S5, r3 and R1 4 (3–5).

Peritreme. Extending setae level of j3.
Venter (Figure 2). Ventral setal pattern 14:JV–3: ZV. Sternal shield smooth, sclerotized, a forked process is visible at posterior margin. The shield with three pairs of setae (ST1, ST2 and ST3) and two pairs of pores (pst1 and pst2); length (ST1–ST3) 54 (53–55), width (distance between setae ST2) 69 (65–73); metasternal setae ST4 and a pair of pores (pst3) on metasternal shields. Genital shield smooth; width at level of genital setae (ST5) 101 (98–105). Ventrianal shield divided into separate ventral and anal shields. Ventral shield bears three pairs of setae (JV1, JV2 and ZV2), the last two setae inserted slightly behind seta JV1. Anal shield with a pair of para-anal (Pa) and a post-anal setae (Pst); and muscle-marks posterolaterally; a pair of crescentic pores (gv3) close the posterior margin of ventral shield and posterior to JV2. Setae JV5 smooth 19 (18–20) in length.
Chelicera (Figure 3). Fixed digit 27 (25–28) long with six apical teeth with pilus dentilis; movable digit 24 (23–25) long with one tooth.
Spermatheca (Figure 4). Calyx narrow, tube-like and long, 35 (33–38); minor duct well developed, long and slightly narrower than the calyx.
Legs (Figure 5). Length of legs (basis of coxae to basis of claws): leg I 436 (430–440); leg II 331 (325–335); leg III 362 (358–368); leg IV 460 (455–465). GeII, III and TiIII each with a knobbed macroseta. Leg IV with three macrosetae all of them also knobbed; GeIV, TiIV, and StIV, 38 (35–40), 32 (30–33) and 26 (25–28) long, respectively. GeII, GeIII and GeIV each with seven setae.
Male (Figures 6–7). (n=3)
Similar to female.
Dorsum. Dorsal setal pattern 10A:9B (r3 and R1 off shield). Dorsal shield oval without waist, strongly sclerotized and smooth, living individuals' dark and brownish in color. The dorsal shield bearing eight pairs of small and rounded solenostomes. Muscle-marks (sigilla) visible mostly on podosoma, length of dorsal shield (j1–J5) 353 (350–355), width (distance between bases of s4) 233 (230–235), width (distance between bases of S2) 234 (240–247). Dorsal setae minute, except for j1 and Z5, which are slightly longer. All dorsal setae smooth. Measurements of dorsal setae as follows: j1 22 (20–24), j3, j4, j5, j6, J2, J5, z2, z4, z5, Z1, Z4, 4 (3–5), Z5 10 (9–12), s4, S2, S4, S5, r3 and R1 4 (3–5).
Peritreme. Extending between setae j3 and z2.
Venter (Figure 7). Ventral setal pattern 11:JV–3, 4:ZV–1,3. Sternogenital shield smooth, sclerotized, with five pairs of setae (ST1, ST2, ST3, ST4 and ST5) and three pairs of crescentic pores. Ventrianal shield divided into separate ventral and anal shields. Ventral shield reticulated; bears three pairs of setae (JV1, JV2 and ZV2) a pair of crescentic pores (gv3) at the posterior margin of ventral shield and posteromedian to JV2; and with four pairs of small pores. Anal shield with a pair of para-anal (Pa) and a post-anal setae (Pst). Setae JV5 smooth 18 (16–19) in length.
Chelicera (Figure 6). Fixed digit 24 (23–25) long with four teeth with pilus dentilis; movable digit 22 (20–23) long with one tooth. Spermatodactyl L-shaped, footlike, 32 (28–35) long, (from basal attachment point to tip of toe).

Legs. Similar to female; leg IV with three macrosetae, all of them knobbed; GeIV, TiIV, and StIV, 36 (34–38), 31 (30–33) and 27 (25–28) long, respectively. GeII, GeIII and GeIV each with seven setae.

Material examined: Mersin: Anamur, Anıtlı town, 20. X. 2008, 4 ♀♀, 1 ♂, 109 m, on *Hedera helix* (Araliaceae) (36° 07' 32.58" N, 32°35'30.19"E); 24. VIII. 2011, 3 ♀♀, 2 ♂♂; 15. VI. 2013 5 ♀♀, 2 ♂♂, all with same collection data.

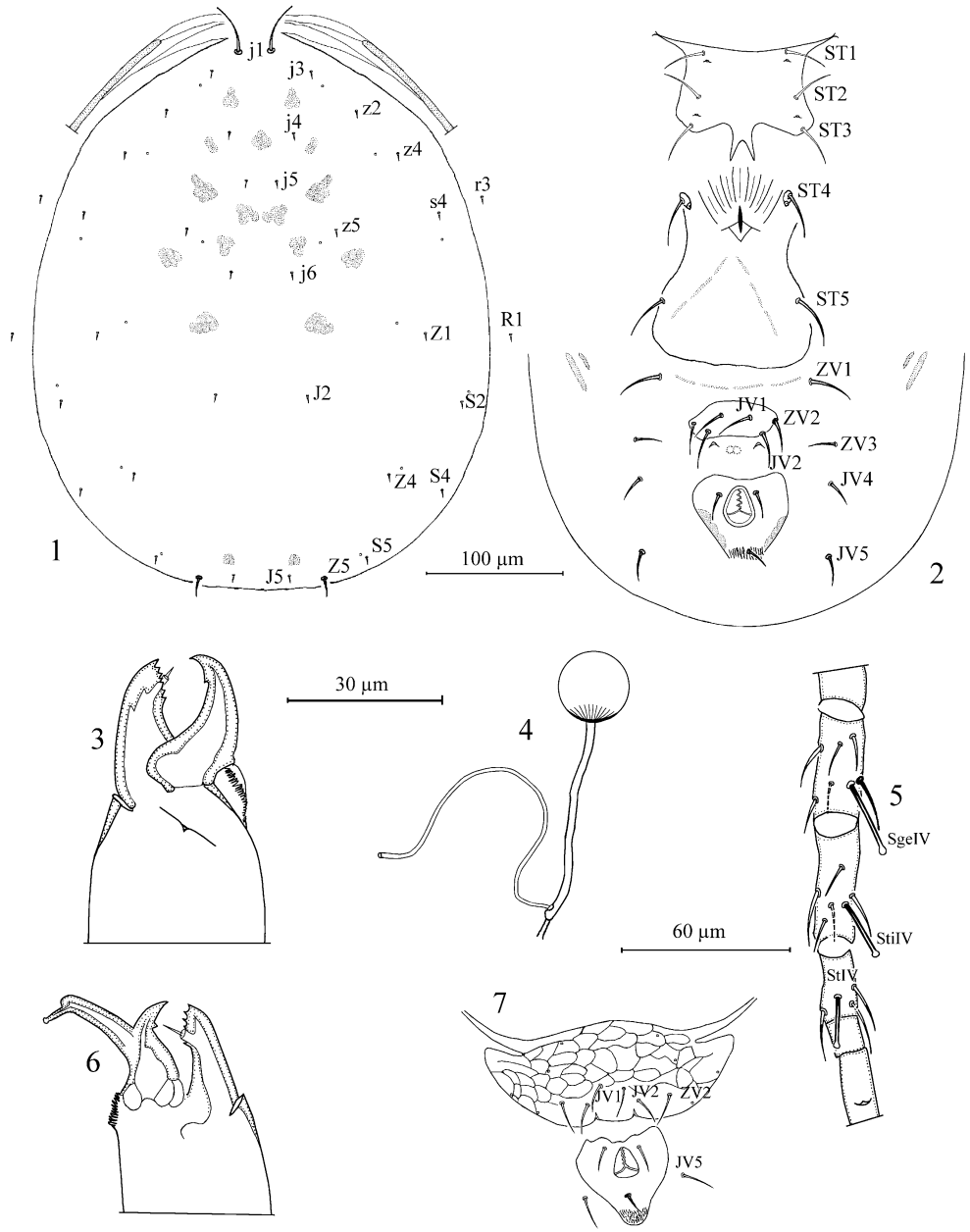

Figure 1-7. *Iphiseius degenerans* (Berlese, 1889), Female: 1. Dorsal shield; 2. Ventral idiosoma; 3. Chelicera; 4. Spermatheca; 5. Leg IV. Male: 6. Chelicera, 7. Ventral and anal shields. Scale bar = 100 µm for 1, 2 and 7; 30 µm for 3, 4; and 6; 60 µm for 5.

Remarks

Morphological characters and measurements of the Turkish specimens are very close to those of re-descriptions (Kolodochka 2006; Moraes et al. 2007; Chant & McMurtry 2007; Papadoulis et al. 2009; Ferragut et al. 2010; Barbar 2013; Denmark & Evans 2011). Moraes et al. (2007) examined several specimens collected from Kenya and found four pairs of preanal setae in three male specimens. As in the other re-descriptions mentioned above, except for Moraes et al. (2007), all males examined from the Turkish population bear three pairs of preanal setae.

Molecular investigations

DNA of *I. degenerans* was successfully amplified using ITS primer pairs. Bands were obtained at ~624bp after amplification of the ITS gene region by gel electrophoresis (Figure 8).

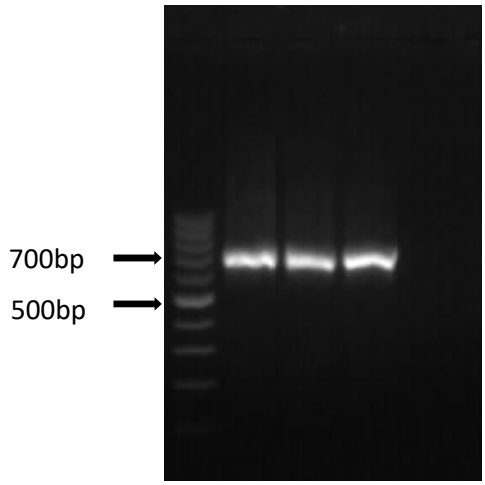

Figure 8. Amplified DNA fragments of *Iphiseius degenerans* using an ITS primer.

The genetic distances among phytoseiid mites, according to the Jukes-Cantor model for their ITS gene regions, are given in Table 1. No genetic divergence was found between the Turkish population and other populations of *I. degenerans*. According to the phylogenetic tree, the Turkish *I. degenerans* population was grouped with the other *I. degenerans* populations. The high bootstrap values at the nodes confirmed this grouping (Figure 9).

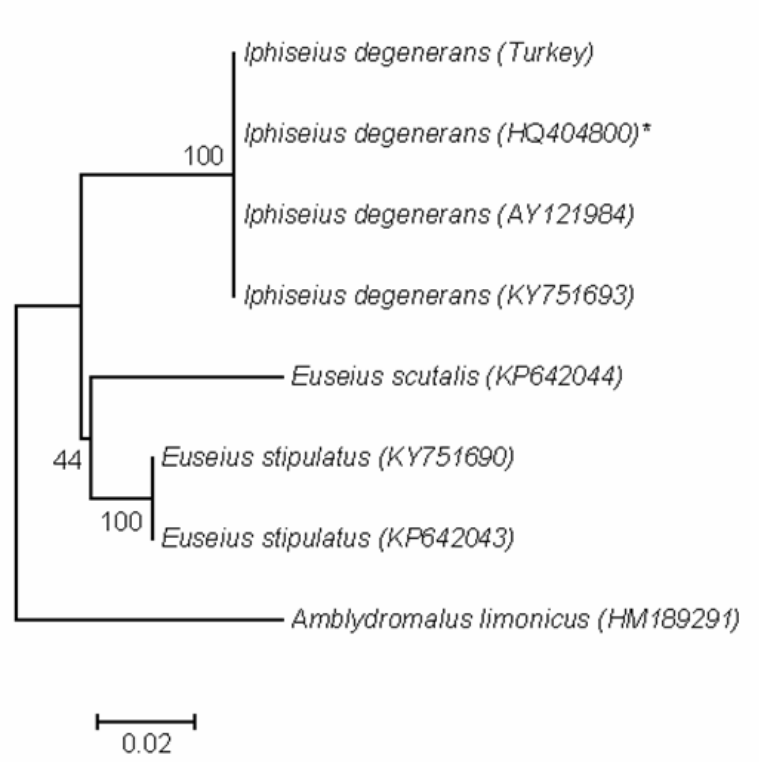

Figure 9. The phylogenetic tree constructed by using the maximum likelihood method based on the Jukes-Cantor model (*Gene bank accession codes are given in parentheses, with the numbers at the nodes indicates the bootstrap values).

Discussion

This study reports morphological and molecular characterization of a Turkish *I. degenerans* population. Our morphological investigation showed no indication of any variations from re-descriptions from other Mediterranean countries, as well as from the USA (Kolodochka 2006; Papadoulis et al 2009; Ferragut et al. 2010, Barbar 2013; Denmark & Evans 2011). This result was confirmed with molecular techniques as we found no genetic divergence between Turkish and other populations of this species, based on ITS sequences.

Table 1. Genetic distances between species of phytoseiid mites, according to the Jukes and Cantor model for the ITS gene region*

	I. degenerans	*I. degenerans*	*I. degenerans*	*I. degenerans*	*E. stipulatus*	*E. stipulatus*	*E. scutalis*	*A. limonicus*
I. degenerans (Turkey)		0.00	0.00	0.00	0.01	0.01	0.00	0.01
I. degenerans (HQ404800)	0.00		0.00	0.00	0.01	0.01	0.00	0.01
I. degenerans (AY121984)	0.00	0.00		0.00	0.01	0.01	0.00	0.01
I. degenerans (KY751693)	0.00	0.00	0.00		0.01	0.01	0.00	0.01
E. stipulatus (KY751690)	0.05	0.05	0.05	0.05		0.00	0.01	0.01
E. stipulatus (KP642043)	0.05	0.05	0.05	0.05	0.00		0.01	0.01
E. scutalis (KP642044)	0.07	0.07	0.07	0.07	0.05	0.05		0.01
A. limonicus (HM189291)	0.10	0.10	0.10	0.10	0.09	0.09	0.11	

*Genetic distances are presented in the lower left and their standard errors in the upper right. Acronyms in parentheses followingspecies names are their accession numbers in the NCBI GenBank database

In this study, we successfully obtained the DNA of *I. degenerans* by using the primer for its ITS gene region. This primer was also successfully used by Santos and Tixier (2017) for DNA amplifications for a wide range of phytoseiid mites. Among them, *Amblyseius andersoni* (Chant), *A. herbicolus* (Chant), *Euseius gallicus* Kreiter and Tixier, *E. scutalis* (Athias-Henriot), *Kampimodromus aberrans* (Oudemans), *Neoseiulus californicus* (McGregor), *Phytoseiulus persimilis* Athias-Henriot, *Phytoseius finitimus* Ribaga, *Typhlodromus* (*Typhlodromus*) *exhilaratus* Ragusa and *T.* (*T.*) *pyri* Scheuten are known from the Turkish fauna (Sekeroglu & Kazak 1993; Çakmak & Çobanoğlu 2006; Faraji et al. 2011; Döker et al. 2014b; Akyazı et al. 2016). Therefore, this primer can be used for molecular characterization of the Turkish Phytoseiidae.

The Phytoseiidae fauna of Turkey is quite rich, with more than 100 species belonging to 21 genera known from the country (Faraji et al. 2011; Döker et al. 2016; Döker 2018). Among them, nine new species were described for the first time, which suggests that many other new species or new reports are still possible (Döker et al. 2014a; 2015; 2017). Each of these known species is a potential biological control agent for spider mites, thrips and whiteflies in IPM programs in Turkish greenhouses. It should be noted that correct identification of these species is the first and the most important step for IPM programs. In this study, the occurrence of *I. degenerans* in Turkey was confirmed by molecular and morphological characters. This study also demonstrated the efficacy of molecular characterization of this

important biological control agent. The results of this study will also be useful for further systematic studies on Turkish Phytoseiidae.

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