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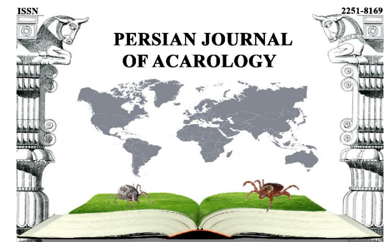

Article

Varroa destructor (Acari: Varroidea) populations from Southern Iran belong to haplotype K of the mitochondrial COI

Zahra Hajializadeh[1], Mahdieh Asadi[1*], Kamal Ahmadi[1] and Asieh Balvasi[2]

1. *Department of Plant Protection, College of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran; E-mail: z.hajializadeh@gmail.com, asadi.mahd@yahoo.com, kahmadi@uk.ac.ir*
2. *Department of Plant Protection, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran; E-mail: asieh balvasi@yahoo.com*

[*] Corresponding authour

ABSTRACT
A molecular characterization based on mitochondrial cytochrome oxidase subunit I gene was used to genotype central-southern Iranian populations of *Varroa destructor* for the first time. A 570 bp segement was amplified by Polymerase Chain Reaction and digested with two restriction enzymes. Digestion of PCR products with *XhoI* produced fragments of 300 and 270 bp in all samples, whereas digestion with *SacI* did not produce any digested fragments. Results suggest that all samples belong to the K haplotype previously described for *V. destructor* in the literature. A total of 22 sequences of Iranian samples (418bp) were registered for the first time in NCBI.

KEY WORDS: Genetic diversity; haplotype; Mesostigmata; mitochondrial genome; molecular characterization.

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INTRODUCTION

Varroa destructor Anderson & Trueman, 2000 is one of the principal agents of weakening apicultures worldwide. Infestation of this pest represents one of the greatest threats to honey bee health, honey production, pollination services and billions of dollars of loss in agricultural crops (Sanford 2001; De la Rúa *et al*. 2009; Genersch *et al*. 2010). Based on mitochondrial DNA (mtDNA) sequences, two distinct haplotypes can be distinguished which are capable of reproducing on its host, *Apis mellifera*: Japanese (J) and Korean (K) genotypes. The J genotype has been reported from Japan, Thailand, Indonesia and some parts of the United States; whereas the K genotype is distributed almost worldwide (Anderson and Trueman 2000) and can be divided into subtypes (K1-1, K1-2, K1-3, K1-4, S1, P1) (Navajas *et al*. 2010; Gajić *et al*. 2013).

Varroa destructor has been present in Iranian apiaries since 1980s (Shahrouzi 2012). Despite the large distribution of apiaries in the country, limited studies have been conducted to evaluate prevalence, pathogenesis and economic loss caused by this mite pest in Iran (Moshaverinia *et al*. 2013; Dadgostar & Nozari 2018). Recent studies on the genetic characterization of this pest have focused the genotype identification of Iranian *V. destructor* based on RAPD markers (Hajializadeh *et al*. 2018). Research was also done by Farjamfar *et al*. (2018) on 28 localities of the North and some scattered parts of Iran where mites were collected and genotyped. These authors sequenced mtDNA fragments from *cox1*, *atp6*, *cox3* and *cytb* genes and showed that all samples were identical

to the K1-1 or the K1-2 haplotypes. In a new sampling effort, additional material was collected from South of Iran from 2015 to 2017 and characterized genetically. In this work, we first used restriction enzyme digestion to identify the haplotype and secondly, we sequenced the PCR products to find the possible differences in nucleotides that we did not find. This paper presents data about mtDNA of southern populations of Iranian *V. destructor* for the first time.

MATERIAL AND METHODS

Mite collection and DNA extraction

Dead *V. destructor* females were collected from 22 localities comprising nine provinces of Southern Iran (Bushehr, Chaharmahal and Bakhtiari, Isfahan, Fars, Kerman, Khuzestan, Sistan and Baluchestan, South Khorasan, Yazd) (Fig. 1). In each location, samples from two to four apiaries were collected. Collected mites were preserved in 70% ethanol and stored at −20 °C. Total DNA was extracted individually from *V. destructor* females using methods described in Anderson and Fuchs (1998). After washing in 70% ethanol, each mite was transferred to a 1.5 mL tube containing 40 mL 2X lysis buffer (120 mg/mL proteinase K, 0.1 M KCl, 0.02 M Tris-HCl, pH 8.3, 5 mM MgCl2, 0.9% Tween 20, 0.9% NP40, and 0.02% gelatin) and grinded. The tubes were incubated two hours at 65 °C, 15 min at 95–100 °C and 5 min at 4 °C. At last centrifuged at 14000g for 5 min. and 20 mL distilled water was added. DNA was stored at −20 °C until PCR amplification.

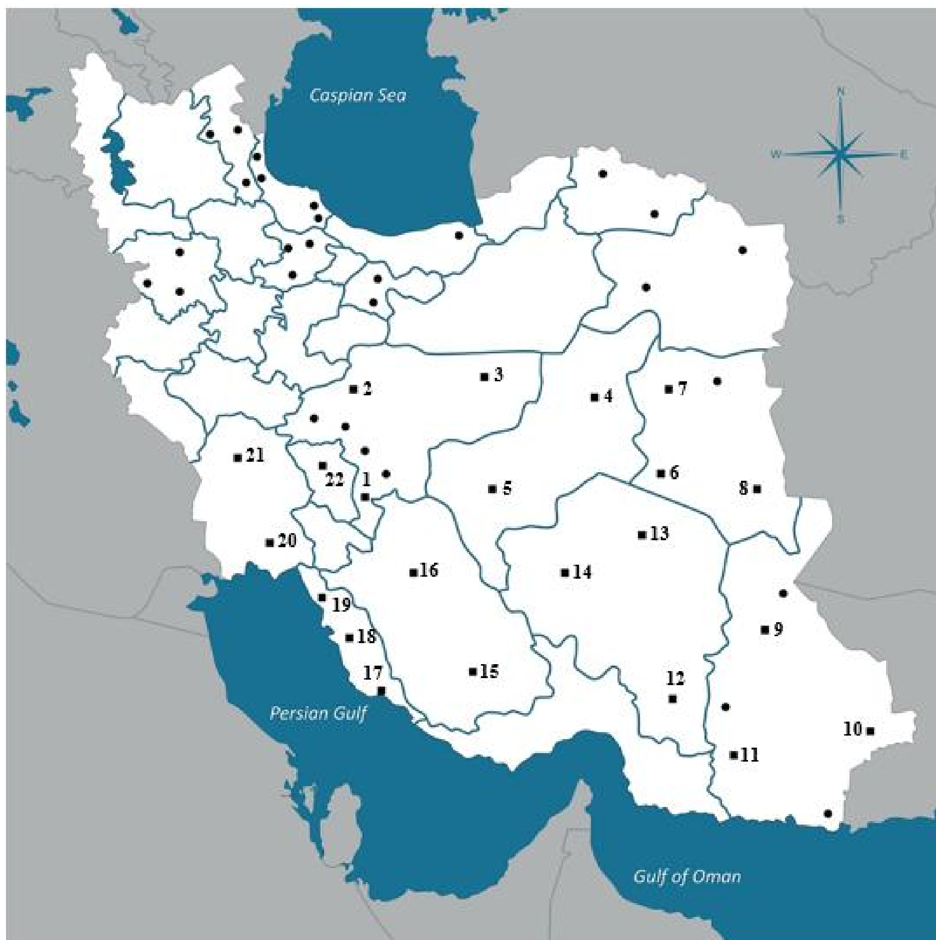

Figure 1. Sampling localities of *V. destructor* in Iran: ● sampling localities by Farjamfar *et al*. (2018), ■ new sampling localities in this study (1.2.3: Isfahan province; 4.5: Yazd province; 6.7.8: South Khorasan province; 9.10.11: Sistan and Baluchestan province; 12.13.14: Kerman province; 15.16: Fars province; 17.18.19: Bushehr province; 20.21: Khuzestan province; 22: Chaharmahal and Bakhtiari province.

PCR

A fragment of the COI gene of *V. destructor* mtDNA was PCR amplified using the primers COXF [5'GG(A/G)GG(A/T)GA(C/T)CC(A/T)ATT(C/T)T(A/T)TATCAAC3'] and COXRa [5'GG (A/T)GACCTGT(A/TA(A/T)AATAGCAAATAC3'] described by Strapazzon *et al.* (2009). Reactions were carried out in 25 µL PCR solution containing 2 µL of the total DNA, 2.5 µL PCR buffer, 0.5 µL 10 mM dNTPs, 1.5 µL 50 mM MgCl2, 1 µL of each primer and 0.3 µL Taq polymerase. Samples were denatured at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min of elongation at 72 °C for 1 min. An extra elongation step at 72 °C for 10 min. was performed. Amplified products were separated by 1% agarose gel electrophoresis.

Restriction enzyme digestion

For haplotype determination, PCR products were digested using two restriction enzymes: *XhoI* and *SacI* (Anderson and Fuchs 1998; Solignac *et al*. 2005). Each 15 µL reaction contained 7 µL of PCR product, 6.5 µL sterile distilled water, 1.5 µL buffer and 0.2 µL of one of the two enzymes, *Xho*I or *Sac*I. Reactions were incubated at 37 °C for 3 h. Products were then placed at 80 °C (*Xho*I) and 65 °C (*Sac*I) for 20 min. to inactivate the enzyme activity and loaded onto 2% TBE agarose gel and electrophoresed at 90 V for 1 h.

Sequencing and data analysis

PCR products of 22 *V. destructor* samples were sequenced at South Korea Makrvzhn company and deposited in GenBank using Bankit software. Obtained sequences were assembled using BioEdit 7.0.9. (Hall 1999), analyzed with MEGA 10.0.4 (Kumar *et al*. 2018) and compared with Korean haplotype (accession No: AF106899) (Anderson and Trueman 2000) and the Japanese haplotype (accession No: AF106897) (Anderson and Trueman 2000) using the BLASTN algorithm.

RESULTS

PCR products generated a fragment of approximately 570 bp in all samples (Fig. 2). Their digestion with the enzyme *Xho*I produced fragments of 300 and 270 bp in all samples (Fig. 3), while digestions with the enzyme *Sac*I, showed no cleavage sites (Fig. 4). These results indicate the presence of only one *V. destructor* haplotype (K) in southern Iran; previously described for *V. destructor* in the literature in other parts of the country.

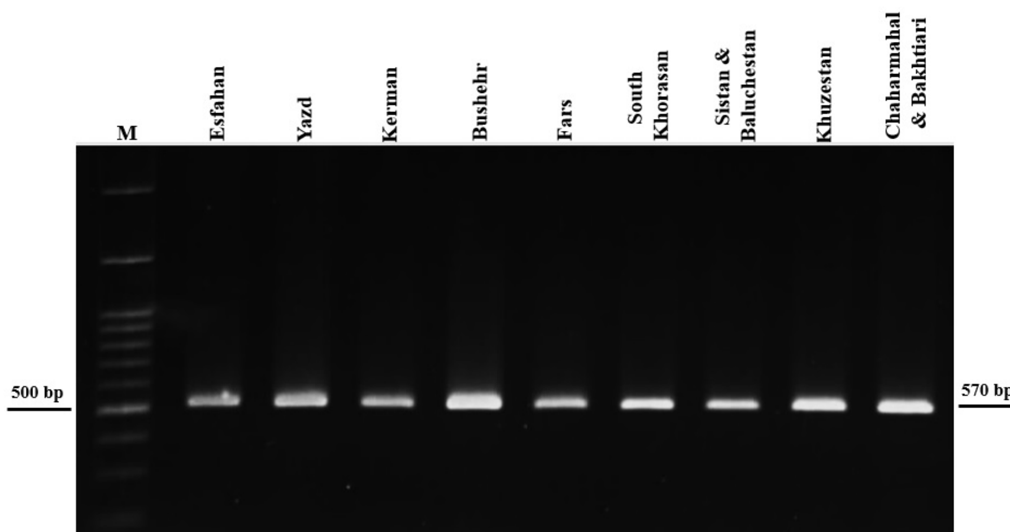

Figure 2. Amplification of COI region of Iranian *V. destructor* mtDNA. M: 100 bp DNA ladder.

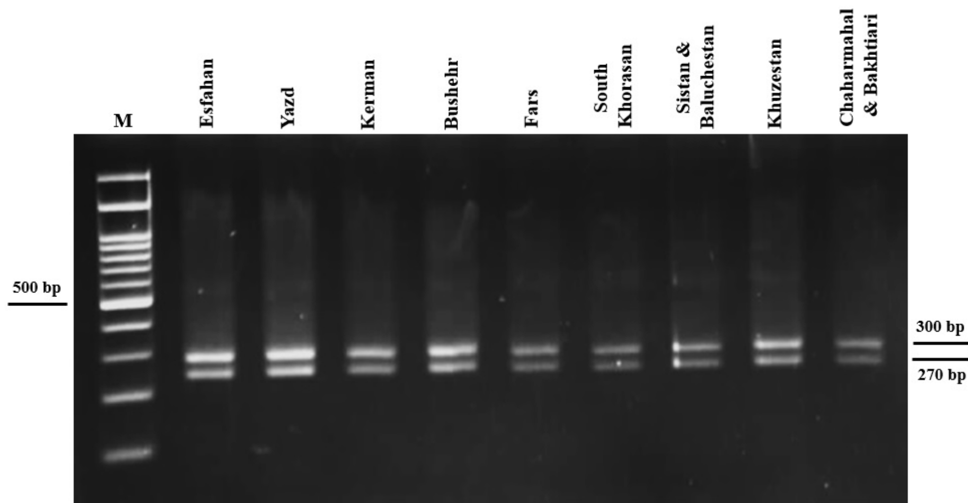

Figure 3. COI region digestion of *V. destructor* mtDNA, by endonuclease *Xho*I. M: 100 bp DNA ladder.

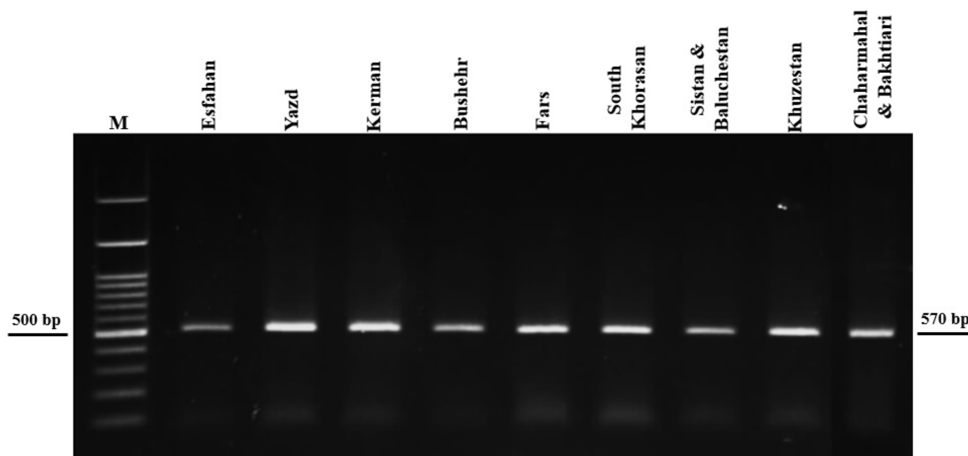

Figure 4. COI region digestion of *V. destructor* mtDNA, by endonuclease *Sac*I. M: 100 bp DNA ladder.

The 22 sequences generated were deposited in GenBank NCBI with the following accession numbers: MH747653.1, MH747654.1, MH747655.1, MH747656.1, MH747657.1, MH747658.1, MH747659.1, MH747660.1, MH747661.1, MH747662.1, MH747663.1, MH747664.1, KU543608.2, KU543609.2, KU543610.2, KU543611.2, KU543612.2, KX375136.2, KX375137.2, KX375138.2, KX375139.2, KX375140.2.

All sequences showed 99% similarity with the COI gene fragment of *V. destructor*, according to sequences available in GenBank NCBI.

DISCUSSION

In the present work, haplotype determination of mite samples from 22 localities based on mtDNA sequence showed that all the mites belong to the K haplotype. Previous study in North of the country based on *cox1*, *atp6*, *cox3* and *cytb* of mitochondrial genes also found the same results (Farjamfar *et al*. 2018). It seems that K is distributed across the country which is in line with the Asian distribution of the K haplotype (Anderson and Trueman 2000).

Finally, according to phylogenetic tree (data not provided) all mite populations of this study belonged to one population and showed no genetic diversity. This lack of genetic diversity is well documented by various studies from other parts of the world (Solignac *et al*. 2005; Muñoz *et al*.

2008; Navajas *et al*. 2010; Kelomey *et al*. 2017). Exceptionally, Hajializadeh *et al*. (2018) reported some little genetic variability of this mite populations based on RAPD marker that can not be used for a sure comparison because of the low numbers of samples in their study and also non-specific characteristic of RAPD marker.

Therefore, despite the usefulness of COI gene variability for separation of the two close species *V. jacobsoni* and *V. destructor*, modern studies concerning genetic diversity should not be based on this mtDNA gene only. Instead, it is advised to analyze concatenated *cox1*, *atp6*, *cox3* and *cytb* mtDNA genes and in a more ideal situation to combine mtDNA data and microsatellite markers introduced by previous researchers (Kelomey *et al*. 2017).

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REFERENCES

Anderson, D. L. & Fuchs, S. (1998) Two genetically distinct populations of *Varroa jacobsoni* with contrasting reproductive abilities on *Apis mellifera*. *Journal of Apicultural Research*, 37: 69–78.

Anderson, D. L. & Trueman, J. W. H. (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology*, 24: 165–189.

Dadgostar, Sh. & Nozari, J. (2018) Classical and geometric morphometric methods reveal differences between specimens of *Varroa destructor* (Mesostigmata: Varroidae) from seven provinces of Iran. *Persian Journal of Acarology*, 7(1): 51–60.

De la Rúa, P., Jaffé, R., Dall'Olio, R., Muñoz, I. & Serrano, J. (2009) Biodiversity, conservation and current threats to European honeybees. *Apidologie*, 40(3): 263–284.

Farjamfar, M., Saboori, A., González-Cabrera, J. & Rodríguez, C.S.H. (2018) Genetic variability and pyrethroid susceptibility of the parasitic honey bee mite *Varroa destructor* (Acari: Varroidae) in Iran. *Experimental and Applied Acarology*, 76(1): 139–148.

Gajić, B., Radulović, Z., Stevanović, J., Kulisić, Z., Vucicević, M., Simeunović, P. & Stanimirović, Z. (2013) Variability of the honey bee mite *Varroa destructor* in Serbia, based on mtDNA analysis. *Experimental and Applied Acarology*, 61: 97–105.

Genersch, E., von der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Büchler, R., Berg, S., Ritter, W., Mühlen, W., Gisder, S., Meixner, M., Liebig, G. & Rosenkranz, P. (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie*, 41: 332–352.

Hajializadeh, Z., Asadi, M. & Kavousi, H. (2018) First report of *Varroa* genotype in western Asia based on genotype identification of Iranian *Varroa destructor* populations (Mesostigmata: Varroidae) using RAPD marker. *Systematic and Applied Acarology*, 23(2): 199–205.

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

Kelomey, A.E., Paraiso, A., Sina, H., Legout, H., Garnery, L. & Baba-Moussa, L. (2017) Genetic characterization of the honeybee ectoparasitic mite *Varroa destructor* from Benin (West Africa) using mitochondrial and microsatellite markers. *Experimental and Applied Acarology*, 72(1): 61–67.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35: 1547–1549.

Moshaverinia, A., Abedi, V. & Safaei, H. (2013) Mite infestation of honey bee (*Apis mellifera*) in apiaries of North East of Iran. *Scientica Parasitologica*, 14(1): 31–35.

Muñoz, I., Garrido-Bailón, E., Martín-Hernández, R., Meana, A., Higes, M. & De la Rúa, P. (2008) Genetic profile of *Varroa destructor* infesting *Apis mellifera* iberiensis colonies. *Journal of Apicultural Research*, 47(4): 310–313.

Navajas, M., Anderson, D.L., de Guzman, L.I., Huang, Z.Y., Clement, J., Zhou, T. & Le Conte, Y. (2010) New Asian types of *Varroa destructor*: a potential new threat for world apiculture. *Apidologie*, 41: 181–193.

Sanford, M.T. (2001) Introduction, spread and economic impact of *Varroa* mites in North America. *In*: Webster, T.C., Delaplane, K.S. (Eds.), *Mites of the honey bee*. Dadant & Sons, Mansfield, OH, pp. 149–162.

Shahrouzi, R. (2012) Three decades of living with *Varroa* in Iran. available at: http://www.apiservices.biz/en/articles/ (Accessed on 10 November 2018).

Solignac, M., Cornuet, J. M., Vautrin, D., Le Conte, Y., Anderson, D., Evans, J., Cros-Arteil, S. & Navajas, M. (2005) The invasive Korea and Japan types of *Varroa destructor*, ectoparasitic mites of the Western honey bee (*Apis mellifera*), are two partly isolated clones. Proceedings of the Royal Society B: Biological Sciences, 272(1561): 411–419.

Strapazzon, R., Carneiro, F. E., Guerra Jr, J.V.C. & Moretto, G. (2009) Genetic characterization of the mite *Varroa destructor* (Acari: Varroidae) collected from honey bees *Apis mellifera* (Hymenoptera, Apidae) in the state of Santa Catarina, Brazil. *Genetics and Molecular Research*, 8(3): 990–997.

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جمعیت‌های *Varroa destructor* (Acari: Varroidea) جنوب ایران به هاپلوتیپ K از سیتوکروم اکسیداز زیرواحد یک میتوکندریایی تعلق دارند

زهرا حاجی علیزاده[1]، مهدیه اسدی[1*]، کمال احمدی[1] و آسیه بلواسی[2]

۱. بخش گیاهپزشکی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران؛ رایانامه‌ها: z.hajializadeh@gmail.com، asadi.mahd@yahoo.com، kahmadi@uk.ac.ir

۲. گروه گیاهپزشکی، دانشکده کشاورزی ، دانشگاه آزاد اسلامی واحد قائم‌شهر، قائم‌شهر، ایران؛ رایانامه: asiehbalvasi@yahoo.com

* نویسنده مسئول

چکیده

ویژگی‌های مولکولی بر اساس ژن سیتوکروم اکسیداز زیرواحد یک میتوکندریایی برای ژنوتیپ جمعیت‌های *Varroa destructor* مرکز-جنوب ایران برای نخستین بار مورد استفاده قرار گرفت. ۵۷۰ جفت باز با واکنش زنجیره‌ای پلیمراز تکثیر و با دو آنزیم برشگر هضم شد. هضم محصول پی سی آر با *XhoI* قطعات ۳۰۰ و ۲۷۰ جفت باز در همه نمونه‌ها تولید کرد، درحالی‌که هضم با *SacI* هیچ قطعات هضم شده‌ای تولید نکرد. نتایج نشان می‌دهد که تمام نمونه‌ها متعلق به هاپلوتیپ K بوده و پیش‌تر برای *V. destructor* در منابع ذکرشده است. در مجموع ۲۲ توالی نمونه‌های ایرانی (418bp) برای نخستین بار در NCBI ثبت شدند.

واژگان کلیدی: تنوع ژنتیکی؛ هاپلوتیپ، میان‌استیگمایان؛ ژنوم میتوکندریایی؛ ویژگی‌های مولکولی.

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