Iranian Journal of Animal Biosystematics (IJAB)
Vol.11, No.1, 79-89, 2015
ISSN: 1735-434X (print); 2423-4222 (online)

Additional records of *Hyalomma marginatum rufipes* Koch, 1844 (Acari: Ixodidae) in southwestern and southern Iran with a molecular evidence

Hosseini-Chegeni, A.[a], Hosseini, R.[a*], Abdigoudarzi, M.[b], Telmadarraiy, Z.[c], Tavakoli, M.[d]

[a] *Department of Plant Protection, Faculty of Agriculture, University of Guilan, Guilan, Iran*
[b] *Razi Vaccine and Serum Research Institute, Department of Parasitology, Reference Laboratory for Ticks and Tick Borne Diseases, Karaj, Iran*
[c] *Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[d] *Lorestan Agricultural and Natural Recourses Research Center, Lorestan, Iran*

(Received: *20 April 2014*; Accepted: *10 February 2015*)

Hyalomma marginatum rufipes (Acari: Ixodidae) along with three closely related subspecies is considered as *marginatum* group. The subspecies had proven as main vector of Crimean Congo hemorrhagic fever virus, the cause of human death in Asia, Africa and Europe. This tick is also a vector of parasitic protozoan *Theileria annulata*, agent of tropical theileriosis in cattle. Nonetheless, taxonomical status of this tick not recognized or confirmed in tick's fauna of Iran, then we decided to show most taxonomic characteristics and confirm the presence of this subspecies in Iran by molecular methods. Tick specimens were collected from cattle in Manujan township, Kerman province, southern Iran. Specimens were identified morphologically using suitable taxonomical identification keys. The morphologically identified specimens were subjected to molecular studies. Morphological and COI gene analysis clearly confirmed the occurrence of *H. m. rufipes* in Iran, however, according to ITS2 fragment *H. m. rufipes* can be the same *H. m. marginatum*. Thus, it seems that based on most popular molecular markers, *H. m. rufipes* and its relative *H. m. marginatum* really should be assigned as a polymorphic species *H. marginatum*.

Key words: *Hyalomma marginatum rufipes*, Ixodidae, *marginatum* group, morphological variation, COI, ITS2, Iran.

INTRODUCTION

The distribution patterns of animal species have a major role in zoogeography that is an essential factor in dispersal and establishing of species across an area (Hickman et al., 2001). Taxonomical, ecological and biological characteristics of a new species have a significant role in further monitoring of species population. Among ticks (suborder Ixodida), species of the genus *Hyalomma* have a great degree of medical and veterinary importance around the world (Sonenshine 2009). They are the specialized vectors of animal and human pathogenic agents, where can be considered as the most excellent choice for biological studies (Bakheit et al., 2012). Unlike, the common behavior of adult *Hyalomma* ticks that infest mammals, it was determined that generally nymphal stages of six

*Corresponding Author: rhosseini@guilan.ac.ir

particular species in this genus feed on the birds and reptiles (Hoogstraal & Aeschlimann 1982). The subspecies *Hyalomma marginatum rufipes* Koch, 1844, commonly named "hairy *Hyalomma*", is considered as an indigenous tick from African continent that can be transported by migratory birds toward distant localities (Hoogstraal 1956, Kaiser et al., 1974). Blood feeder immature stages of *H. m. rufipes* can remain more than 16 days on the bird host body, where they have an adequate time to spread across more farther distances (Korch 1994). *H. m rufipes* is closely related to *H. m. marginatum*, *H. m. turanicum* and *H. m. isaaci*, all recently promoted to species level (Apanaskevich & Horak 2008, Guglielmone et al., 2014). This subspecies has been reported as a main vector of Crimean Congo hemorrhagic fever virus (CCHFV), a human fatal disease in Asia, Africa and Europe (Hoogstraal 1979). This tick is also a vector of parasitic protozoan *Theileria annulata*, the agent of tropical theileriosis in cattle (d'Oliveira et al., 1997). Nonetheless, taxonomical status of this tick not recognized or confirmed in tick's fauna of Iran (Mazlum 1971, Filippova et al., 1976, Rak 1976, Hoogstraal & Valdez 1980, Hashemi-Fesharaki et al., 2002, Rahbari et al., 2007, Nabian et al., 2009, Razmi & Ramoon 2012). However, Hoogstraal in 1967 (cited in Abdigoudarzi 2004) compiled a taxonomical key to the identification of *marginatum* group members (*H. m. marginatum*, *H. m. rufipes* and *H. m. turanicum*) in Iran. In an comprehensive study on *Hyalomma* fauna of Iran few specimens *H. rufipes* recognized by the first author in Lorestan and Hormozgan provinces (Hosseini-Chegeni et al., 2013). Then, we decided to recording more specimens of this rare and important subspecies as well as showing most taxonomic characteristics and confirming the presence of this subspecies in Iran by molecular methods. The molecular identification of animal taxa using DNA barcoding recommended by different authors (Hebert et al., 2003, Tautz et al., 2003). The basis of DNA barcoding is cytochrome oxidase subunit I (COI or COXI) which is reported as a choice gene for the identification of tick species (Lv et al., 2014, Zhang & Zhang 2014). Internal transcribed spacer 2 (ITS2), as well as, is the best choice for the identification of ticks because of the low level intraspecies variability and high level interspecies differences (Rumer et al., 2011).

MATERIALS AND METHODS

Tick collection

Ten specimens were collected from Chahshoor village, Manujan township, Kerman province, southern Iran (27°23'33"N/57°32'05"E) and an unknown region in Khuzestan province. The male specimens were collected from cattle. The identified subspecies are preserved in the tick's collection of Natural History Museum of University of Guilan.

Morphological studies

Determination of specimens

The specimens were identified using suitable taxonomical identification keys including Delpy (1949a,b), Feldman-Muhsam (1954), Hoogstraal (1956), Abdigoudarzi (2004), Walker et al (2007) and Apanaskevich and Horak (2008). The characters were used to the identification of subspecies shown in Table 1.

Drawing

Taxonomical characters of subspecies were drawn by drawing tube (Olympus SZH-Japan) connected to stereomicroscope (Olympus®) and then, redrawing using Corel Draw Graphics Suite® (version X6), software.

TABLE 1. A set of taxonomical characters for differentiation male *Hyalomma marginatum* group in the Iranian specimens according to Delpy (1949b), Walker et al., (2007), Apanaskevich and Horak (2008), Hosseini and Tavakoli (2012).

Character	Character state	Taxon diagnosed
Scutum punctuation density	Sparse	*H. m. marginatum*
	localized-regular	*H. m. turanicum*
	dense/very dense	*H. m. rufipes*
Scutum color	dark-light brown	*H. m. marginatum*
	dark-dark red	*H. m. turanicum*
	dark-dark brown	*H. m. rufipes*
Scutum shape	-	*H. m. marginatum*
	-	*H. m. turanicum*
	egg shaped	*H. m. rufipes*
Posterior scutal margin shape	-	*H. m. marginatum*
	-	*H. m. turanicum*
	Round	*H. m. rufipes*
Spiracular plate tail*	Broad	*H. m. marginatum*
	Narrow	*H. m. turanicum*
	very narrow	*H. m. rufipes*
Circumspiracular integument setae density	Sparse	*H. m. marginatum*
	moderately dense	*H. m. turanicum*
	very dense	*H. m. rufipes*
Legs bands	Present	*H. m. marginatum*
	Present	*H. m. turanicum*
	Present	*H. m. rufipes*
Lateral grooves	long**	*H. m. marginatum*
	Long	*H. m. turanicum*
	short or long-concealed	*H. m. rufipes*
Posteromedian, paramedian grooves and caudal field	shallow and distinct	*H. m. marginatum*
	superficial and poorly developed	*H. m. turanicum*
	absent and indistinct punctuated	*H. m. rufipes*

-: No characteristic, *: Joint of body and tail of spiracular plate, **: Sometimes continuing towards eyes as lines of punctuations

Molecular studies

DNA extraction

DNA was extracted by phenol-chloroform method according to Sambrook and Russell (2001) with some modifications.

Primers

The list of primers used in the present study was shown in Table 2. Also, primer TRH was designed, because the failures in amplification of ITS2 fragment *Hyalomma* species using universal primers (http://sites.biology.duke.edu/fungi/mycolab/primers.htm).

TABLE 2. Primers used in the present study.

Target	Name	Type	Primer sequence (5′→3′)	Reference
COI	C1-J-1718	Forward	GGAGGATTTGGAAATTGATTAGTTCC	Simon et al., (1994)
	C1-N-2191	Reverse	CCCGGTAAAATTAAAATATAAACTTC	Simon et al., (1994)
ITS2	3SA	Forward	CTAAGCGGTGGATCACTCGG	Barker (1998)
	TRH	Reverse	TCTTCGGGACGGCGACTG	Designed

Polymerase chain reaction

PCR was carried out at thermal cycler MyGenie® (Bioneer, South Korea) and Bio-Rad® (U.S.) for all specimens. Temperature profiles for amplification of COI and ITS2 gene, as well as, the required ingredients for each PCR reaction are shows in Table 3 and
Table **4**, respectively.

TABLE 3. Temperature profiles used for amplification of COI and ITS2 gene.

	Step	Temperature (°C)	Time (minute)
COI	1. Initial denaturation	95	5
	2. Denaturation	94	1
	3. Annealing	54	1
	4. Extension	72	1
	Repeat steps 2-4, 34 times		
	5. Final extension	72	10
ITS2 (touchdown)	1. Initial denaturation	95	5
	2. Denaturation	94	1
	3. Annealing	65-45*	1
	4. Extension	72	1.30
	Repeat steps 2-4, 20 times		
	5. Denaturation	94	1
	6. Annealing	45	1
	7. Extension	72	1.30
	Repeat steps 5-7, 20 times		
	8. Final extension	72	10

*: Temperature may be decrease 1° C/cycle to 45° C

TABLE 4. The quantity and concentration of PCR ingredients.

Ingredient (company-initial concentration)	Quantity (µl)	Final concentration (for 25 µl)
Sterile water	14.8	14.8 µl
PCR buffer (Bioflux®-10x)	2.5	1 µl
$MgCl_2$ (Bioflux®-50 mM)	1*	2 mM
Forward primer (10 µM)	1	0.4 µM
Reverse primer (10 µM)	1	0.4 µM
dNTPs (Bioflux®-10 mM)	0.5	200 µM
Taq DNA polymerase enzyme (Bioflux®-5 U/µl)	0.3	1.5 U
gDNA template	4	Not measured

*: The amount $MgCl_2$ can be increase to 2 µl

Gel electrophoresis, purification and sequencing

The PCR products were visualized by 1% agarose gel electrophoresis. PCR products with desired band were purified using GeneJET Gel Extraction Kit®, then were sent to Sequetech® Company for sequencing using ABI 3730XL DNA sequencer.

BLAST of sequences, calculation distance difference and construction of phylogenetic tree

The results of COI and ITS2 sequencing were reviewed by Finch TV® software and edited manually. The Basic Local Alignment Search Tool (BLAST) was used to compare the similarity of our sequences with sequences presented in GeneBank database (http://www.ncbi.nlm.nih.gov/blast). The sequences were submitted to GeneBank with accession numbers KP219873 and KP208963 for COI and ITS2, respectively (released in 2016). The pairwise distance differences were computed using the Kimura 2-parameter method (Kimura 1980) by MEGA6® software (Tamura et al., 2013). As well as, a phylogenetic tree for each gene was constructed using Neighbor-Joining (NJ) method

by MEGA6 software. The evolutionary distances were computed using the Maximum Composite Likelihood method.

RESULTS

Morphology

The specimens were diagnosed as *Hyalomma marginatum rufipes* Koch, 1844 based on scutal punctuation, central festoon and other taxonomical characters presented in the earlier studies. The scutal patterns of *H. m. rufipes* collected from different area of Iran represented in Figure 1 and Figure 2. A particular variation observed as a punctate scutum with glabrous area (Figure 2, 1-III).

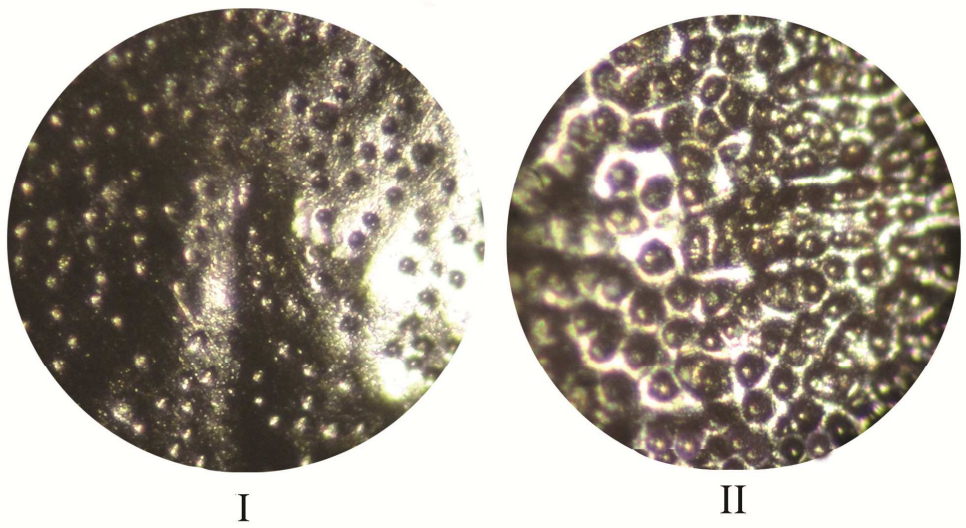

FIGURE 1. Two scutal punctuation pattern as a section in mid scutum *Hyalomma marginatum rufipes* I) small and distant, II) large and compact.

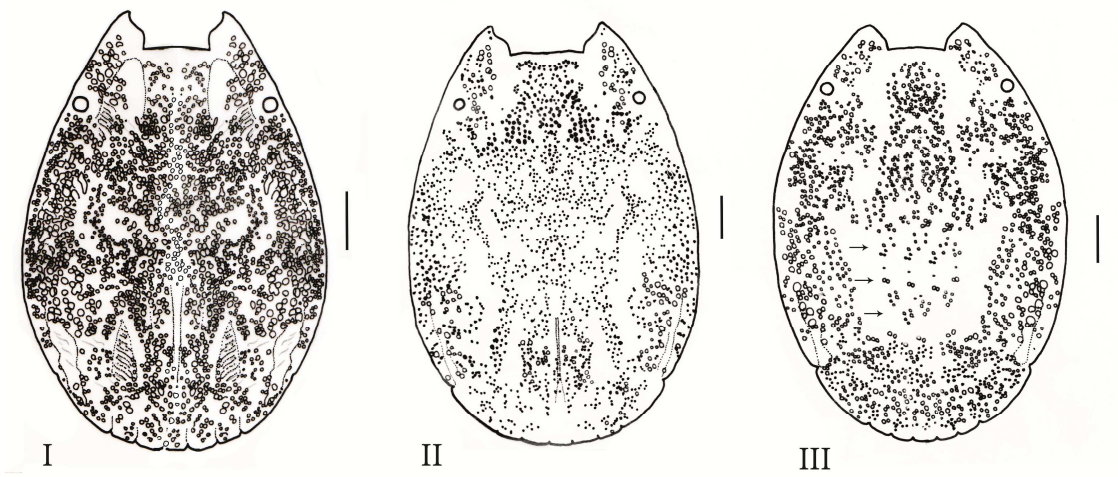

FIGURE 2. Variation in punctuation of whole scutum *H. rufipes*; I: very dense and compact (Kerman specimen), II) dense, small and distant (Khuzestan specimen), III) dense, small and distant with glabrous area (arrow) (Khuzestan specimen).

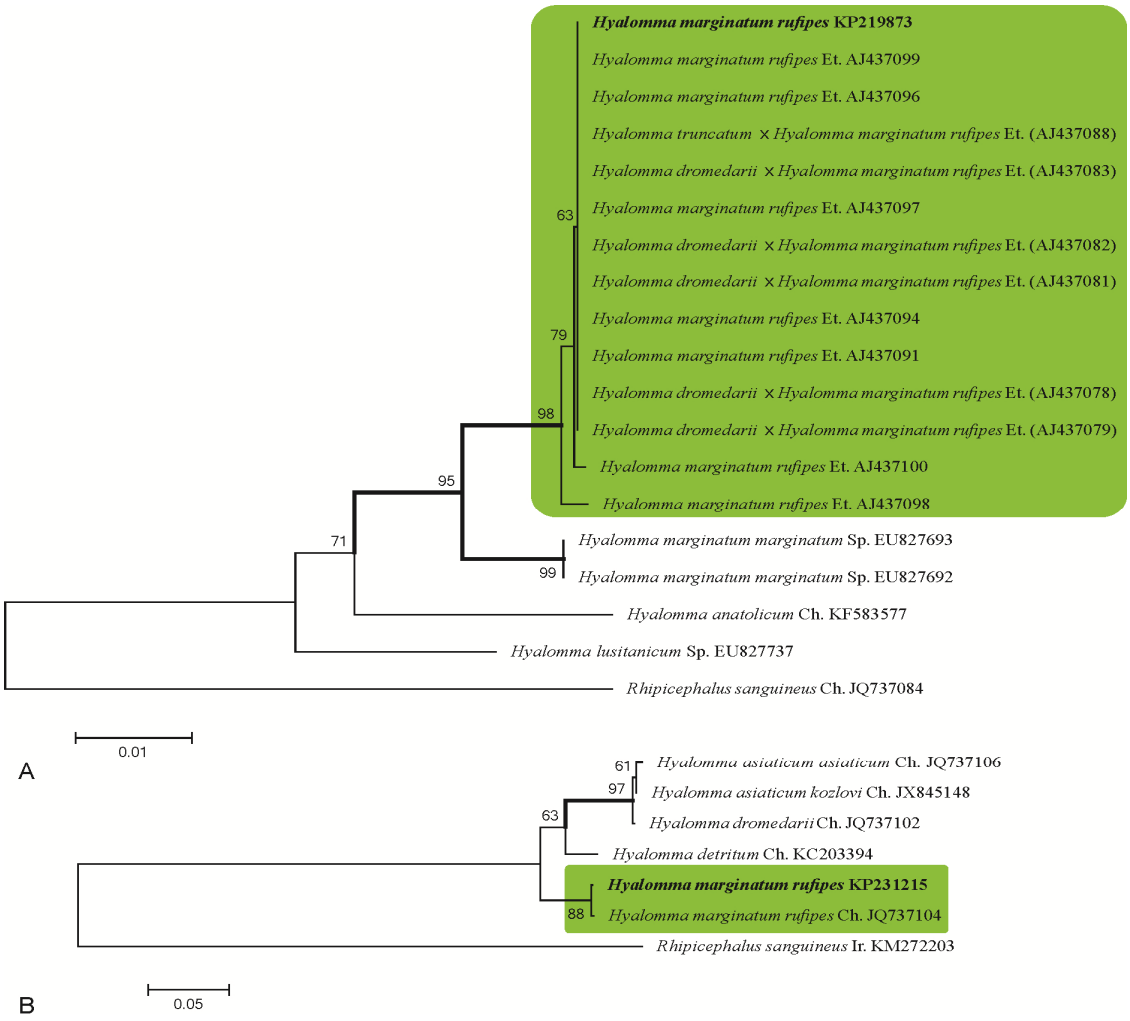

FIGURE 3. Phylogenetic trees reconstructing evolutionary relationship among *H. marginatum* group and other *Hyalomma* species, phylogenetic tree of COI (A) and ITS2 (B) sequences data using NJ method with 1000 replicates bootstrapping, green boxes representing a clade including sequence of this study (as bold) and GenBank sequences with accession number and abbreviated country name, taxon *Rhipicephalus sanguineus* is taken as outgroup, thicker branches showing bootstrap value greater than 90%, × sign (A) indicating a morphologically hybrid or intermediate taxa were stated in Rees et al., (2003), Ch. China, Et. Ethiopia, Ir. Iran, Sp. Spain.

Molecular essay and phylogenetic tree

The result of BLAST showed the COI sequence of *H. m. rufipes* has 99% similarity with COI sequences of *H. m. rufipes* (accession numbers: AJ437091, AJ437094, AJ437096-100), *H. truncatum* (accession number: AJ437088) and *H. dromedarii* (accession numbers: AJ437078-79, AJ437081-83), as well as, 96% similarity with COI sequences of *H. m. marginatum* from Spain (accession numbers: EU827692-93). The result of BLAST with partial ITS2 showed 99% identity with a single sequence belongs to *H. m. rufipes* from China (accession number: JQ737104). As well as, the comparison ITS2 sequence of *H. m. rufipes* of this study with an ITS2 sequence *H. m. marginatum* submitted from Iran (accession number: FJ416322) shown 93% similarity. Both COI and ITS2 phylogenetic trees along with constructed clades is shown in Figure 3. The phylogenetic analysis were conducted on 19

nucleotide sequences with 437 positions and 7 nucleotide sequences with 583 positions in the final dataset for COI and ITS2 phylogenetic trees, respectively. All gap positions and missing data were removed.

DISCUSSION

Traditional taxonomy

Hyalomma marginatum rufipes is a member of *marginatum* group. This subspecies have been considered as a tick dispersed via land migratory birds and it can established outside its original distribution, then causes the spread of CCHF virus to the new localities (Hoogstraal & Kaiser 1958a, Hoogstraal 1972, Kaiser et al., 1974). Leastwise, ten forms of migrant birds that can transport immature stages of *H. m. rufipes* into Egypt, clearly are responsible for introducing of *H. m. rufipes* to new areas (Hoogstraal & Kaiser 1958b). Since morphological characters of ticks of *marginatum* group are more similar and qualitative, thus their taxonomical status is debatable and species delimitation not to be easy (Apanaskevich & Horak 2008). It is thought that *H. m. rufipes* has been introduced to Iran by land migratory bird or imported livestock, thus the report of this subspecies from Iran is more important since it has been reported originally from Ethiopian region (Egypt, Libya, Palestine, Anatolia) and southern Russia in small numbers (Hoogstraal & Kaiser 1958a). In the current study three taxonomical characters including scutal punctuation, circumspiracular integument setae and spiracular plate tail were seen as the most valuable characters (i.e., less variables morphological traits) using for differentiating of male specimens *H. m. rufipes*. A unique trait that was found for identification of three subspecies *rufipes*, *marginatum* and *turanicum* is breadth of spiracular plate tail at the junction tail to spiracle body. This character in *rufipes* is narrower than other taxa and properly illustrated in Pomerantzev (1950), however, it has been called as *H. plumbeum impressum*. Feldman-Muhsam and Kahn (1958) compared laboratory progeny of a single female *H. rufipes* and reported notable variability in the characteristics e.g., scutal punctuation, total shape and color of scutum. They showed less variability in circumspiracular integument setae (Feldman-Muhsam & Kahn 1958). Whereas, interbreeding among populations of three subspecies *rufipes*, *marginatum* and *turanicum* occur in areas where the migratory birds transfer hybrid forms into other region (Hoogstraal et al., 1963). Thus, intermediate forms combining form two or three subspecies *rufipes*, *marginatum* and *turanicum* may be seen. Delpy (1949b) splits the taxon *H. rufipes* as two subspecies *H. r. rufipes* and *H. r. glabrum* based on total scutum shape, posterior margin of scutum and circumspiracular integument setae density. However, these characters are very debatable and ambiguous for the differentiation of species as we examined many specimens that shown the combined characteristics of two subspecies described by Delpy (1949b).

Molecular phylogeny

According to COI phylogenetic tree, *H. m. rufipes* has a subspecific taxonomical level. Thus, it seems that based on COI, a protein coding gene, two taxon of *rufipes* and *marginatum* really should be assigned as distinct subspecies of polymorphic species *H. marginatum* (2% pairwise distance difference). So that, both *rufipes* and *marginatum* shared a common ancestor in COI phylogenetic tree. Unfortunately, except an ITS2 sequence submitted from China (JQ737104) further sequences were not found in GenBank to comparing our *H. m. rufipes* ITS2 sequence, exactly. However, on the ITS2, a non protein coding gene, *H. m. rufipes* is a distinct subspecies. Based on COI phylogenetic tree and 4% pairwise distant difference, *H. m. rufipes*, *H. anatolicum* and *H. lusitanicum* are closely related species, phylogenetically. This difference among ITS2 sequences of *H. m. rufipes*, *H. detritum* and the *H. asiaticum* group is 6-9%, greater than COI sequences. Rees et al., (2003) reported some hybrid forms in *marginatum* group (used in COI phylogenetic tree) representing the probability of

crossbreeding among *Hyalomma* species. According to maternal inheritance of mitochondrial genome (e.g., COI gene), thus COI phylogeny is reflecting of only female species coupled with male.

ACKNOWLEDGMENTS
Authors are grateful to M. Paydareh and H. Bahrani for collecting tick specimens in Kerman and Khuzestan provinces, respectively. Also, from F. Shirzaiiyan-Zarouni for her encouragements as well as H. Hosseini for his kindness helps.

LITERATURE CITED

Abdigoudarzi, M. 2004. Review of Ticks (Acari: Ixodidae) and redescription of Hyalomma and Rhipicephalus genera by RAPD-PCR in Iran. Doctor of Philosophy, Department of Agricultural Entomology, Tarbiat Modares University. Tehran Iran.

Apanaskevich, D.A., Horak, I.G. 2008. The genus *Hyalomma* Koch, 1844.V. Re-evaluation of the taxonomic rank of taxa comprising the *H.* (*Euhyalomma*) *marginatum* Koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology. *International Journal of Acarology* 34, 13–42.

Bakheit, A.M., Latif, A.A., Vatansever, Z., Seitzer, U., Ahmed, J. 2012. The huge risks due to *Hyalomma* ticks. In: Mehlhorn H, ed. Arthropods as Vectors of Emerging Diseases (Parasitology Research Monographs). Springer-Verlag, Berlin Germany, pp. 167–94.

Barker, S.C. 1998. Distinguishing species and populations of Rhipicephaline ticks with ITS 2 ribosomal RNA. *Journal of Parasitology* 84, 887–92.

d'Oliveira, C., Van der Weide, M., Jacquiet, P., Jongejan, F. 1997. Detection of *Theileria annulata* by the PCR in ticks (Acari: Ixodidae) collected from cattle in Mauritania. *Experimental and Applied Acarology* 21, 279–91.

Delpy, L.P. 1949a. Essai critique de synonymie du genre *Hyalomma* C. L. Koch 1844 depuis Linné, 1758. *Annales de Parasitologie Humaine et Comparée* 24, 464–94.

Delpy, L.P. 1949b. Révision par les voies expérimentales du genre *Hyalomma* C. L. Koch, 1884. 2^{e} partie. *Annales de Parasitologie Humaine et Comparee* 24, 97–109.

Feldman-Muhsam, B. 1954. Revision of the genus *Hyalomma* I. Description of Koch's types. *Bulletin of Research Council of Israel* 4, 150–70.

Feldman-Muhsam, B., Kahn, J. 1958. The variation in laboratory bred ticks. *Journal of Parasitology* 44, 23.

Filippova, N.A., Neronov, V.M., Farhang-Azad, A. 1976. Data on ixodid tick fauna (Acarina, Ixodidae) of small mammals in Iran. *Entomologicheskoe Obozreniye* 55, 467–79.

Guglielmone, A.A., Robbins, R.G., Apanaskevich, D.A., Petney, T.N., Estrada-Peña, A., Horak, I.G. 2014. The hard ticks of the world (Acari: Ixodida: Ixodidae). Springer, Dordrecht Netherlands, pp. 738.

Hashemi-Fesharaki, R., Abdigoudarzi, M., Esmaeil-Nia, K. 2002. An illustrated guide to the Ixodidae ticks of Iran. Veterinary Organisation of Iran, Tehran Iran [In Persian], pp. 110.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R. 2003. Biological identifications through DNA barcodes. *Proceeding Royal Society of London* Serie B 270, 313–21.

Hickman, C.P., Roberts, L.S., Larson, A. 2001. Integrated principles of zoology. McGraw-Hill, New York USA, pp. 899.

Hoogstraal, H. 1956. African Ixodoidea. I. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary reviews of the genera *Boophilus*, *Margaropus* and *Hyalomma*), research report. United State Navy, Washington DC, pp. 1101.

Hoogstraal, H. 1972. Birds as tick hosts and as reservoirs and disseminators of tickborme infectious agents. *Wiadomosci Parazytologiczne* 18, 703–06.

Hoogstraal, H. 1979. The epidemiology of tick-borne Crimean Congo haemorrhagic fever in Asia, Europe, and Africa. *Journal of Medical Entomology* 15, 307–417.

Hoogstraal, H., Aeschlimann, A. 1982. Tick host specificity. *Bulletin de la Société Entomologique Suisse* 55, 5–32.

Hoogstraal, H., Kaiser, M.N. 1958a. Observations on Egyptian *Hyalomma* ticks (Ixodoidea, Ixodidae). 2. parasitism of migrating birds by immature *H. rufipes* Koch. *Annals of the Entomological Society of America* 51, 12–16.

Hoogstraal, H., Kaiser, M.N. 1958b. The ticks (Ixodoidea) of Egypt: A brief review and keys. The *Journal of the Egyptian Public Health Association* 33, 51–85.

Hoogstraal, H., Kaiser, M.N., Traylor, M.A., Guindy, E., Sobhy, G. 1963. Ticks (Ixodidae) on birds migrating from Europe and Asia to Africa, 1959-61. *Bulletin World Health Organization* 28, 235–62.

Hoogstraal, H., Valdez, R. 1980. Ticks (Ixodoidea) from wild sheep and goats in Iran and medical and veterinary implications. *Fieldiana Zoology* 6, 1–16.

Hosseini-Chegeni, A., Hosseine, R., Tavakoli, M., Telmadarraiy, Z., Abdigoudarzi, M. 2013. The Iranian *Hyalomma* (Acari: Ixodidae) with a key to the identification of male species. *Persian Journal of Acarology* 2, 503–29.

Hosseini, A., Tavakoli, M. 2012. A taxonomic study on hard ticks *Hyalomma marginatum* species group (Acari: Ixodidae) in Iran. Proceeding of 1st International & 8th National Congress of Parasitology & Parasitic Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran.

Kaiser, M.N., Hoogstraal, H., Watson, G.E. 1974. Ticks (Ixodoidea) on migrating birds in Cyprus, fall 1967 and spring 1968, and epidemiological considerations. *Bulletin Entomological Research* 64, 97–110.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16, 111–20.

Korch, G.W. 1994. Geographic dissemination of tick-borne zoonoses. In: Sonenshine DE, Mather TN, eds. Ecological dynamics of tick-borne zoonoses. Oxford University Press, Oxford UK, pp. 139–97.

Lv, J., Wu, S., Zhang, Y., Chen, Y., Feng, C., Yuan, X. Jia, G., Deng, J., Wang, C., Mei, L., Lin, X. 2014. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasites & Vectors* 7, 1–12.

Mazlum, Z. 1971. Ticks of domestic animals in Iran: geographic distribution, host relation, and seasonal activity. *Journal of Veterinary Faculty University Tehran Iran* 27, 1–32 [In persian].

Nabian, S., Rahbari, S., Changizi, A., Shayan, P. 2009. The distribution of *Hyalomma* spp. ticks from domestic ruminants in Iran. *Medical and Veterinary Entomology* 23, 281–83.

Nicholas, K.B., Nicholas, H.B.J., Deerfield, D.W. 1997. GeneDoc: analysis and visualization of genetic variation. *Embnew News* 4, 14.

Pomerantzev, B.I. 1950). Fauna of USSR arachnida: Ixodid ticks (Ixodidae), Fauna SSSR. Akademii Nauk SSSR, Leningrad USSR, pp. 224 [In Russian].

Rahbari, S., Nabian, S., Shayan, P. 2007. Primary report on distribution of tick fauna in Iran. *Parasitology Research* 101 S175–S77.

Rak, H. 1976. Tick borne diseases and their vectors in Iran. Proceeding of International Congress on Tick-Borne Diseases and Their Vectors, Edinburgh UK.

Razmi, G.R., Ramoon, M. 2012. A study of tick fauna in Tandoureh national park, Khorasan Razavi province, Iran. *Acarina* 20, 62–65.

Rees, D.J., Dioli, M., Kirkendalla, L.R. 2003. Molecules and morphology: evidence for cryptic hybridization in African *Hyalomma* (Acari: Ixodidae). *Molecular Phylogenetics and Evolution* 27, 131–42.

Rumer, L., Sheshukava, O., Dautel, H., Danosa-Mantke, O., Niedrig, M. 2011. Differentiation of medically important Euro-Asian tick species *Ixodes ricinus*, *Ixodes persulcatus*, *Ixodes hexagonus*, and *Dermacentor reticulatus* by polymerase chain reaction. *Vector-Borne and Zoonotic Diseases* 11, 899–905.

Sambrook, J., Russell, D.W. 2001. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, New York USA, pp. 737.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* 87, 651–701.

Sonenshine, D.E. 2009. Ticks. In: Resh VH, Cardé RT, eds. Encyclopedia of insects. Academic Press, San Diego CA, pp. 1003–11.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30, 2725–29.

Tautz, D., Arctander, P., Minelli, A., Thomas, R.H., Vogler, A.P. 2003. A plea for DNA taxonomy. *Trends in Ecology & Evolution* 18, 70–74.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Peña, A., Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M. 2007. Ticks of domestic animals in Africa: a guide to identification of species. *Bioscience Reports*, Edinburgh UK, pp. 221.

Zhang, R.L., Zhang, B. 2014. Prospects of using DNA barcoding for species identification and evaluation of the accuracy of sequence databases for ticks (Acari: Ixodida). *Ticks and Tick-borne Diseases* 5, 352–58.