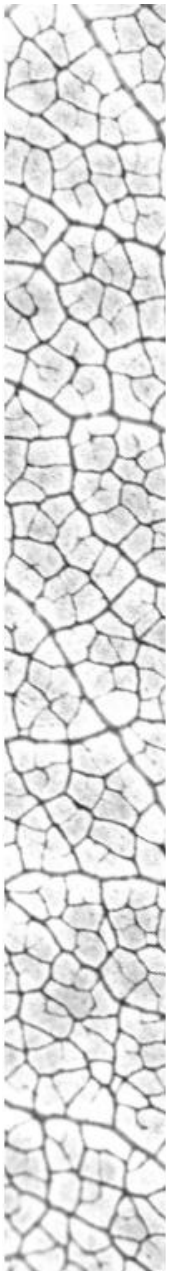

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Genetic diversity among pishdar dog in sulaimani governorate using RAPD-PCR technique

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Abstract
Present study aimed to characterization Pishdar shepherd dogs which is a native breed specified in Kurdistan- Iraq. For this purpose the RAPD markers were used to study genetic diversity among nine geographical locations in Suliamani governorate. A total of 74 samples were typed using twenty RAPD primers. Moreover, fourteen out of the twenty primers had clear bands. A total of 709 bands were scored, of which 57 bands (51.48%) were polymorphic and 15 of polymorphic band were unique bands. For all regions, Nei's gene diversity, Shannon index, percentage of polymorphic loci and unique bands are in the range of 0.19 to 0.49, 0.34 to 0.68, 10 to 100, and 1 to 5, respectively. The UPGMA dendrograms showed three clusters, the 1st cluster branch consisted of the Sitak and Halabja, the 2nd cluster was include both of the Qala-Diza and Rania and the 3rd one included constitutes four sub-clumps the 1st branch consist of (Dokan and Suliamani) region, the 2nd branch harbored the Huwana region only. The 3rd one covers the Sangasar region. Finally, the 4th sub-cluster possesses the Pishdar group. The results indicated that impressive logical result, showed low genetic distance between the Dokan and Suliamani population, in addition to small genetic distance between Qala-Diza and Rania, and moderate genetic distance between Sitak and Halabja. Which means there was no genetic variation in between these populations according to the near geographical distance between these areas. Thus, the inbreeding mating among these areas records high value. Meanwhile, the Huwana, Sangasar and Pishdar sub-clusters population documented a moderate genetic distance between them. Nevertheless, the high genetic distance that recorded (56.13%) among the region's population of Pishdar dog showed ample ground for mating within this breed in suliamani province.

Keywords: RAPD-PCR, pishdar dog, polymorphism, genetic distance

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INTRODUCTION

Archaeological discoveries display the presence of domestic dogs in Germany, Palestine, and North Iraq currently Kurdistan region at 14,000-16,000 years before the present (BP) (Clutton-Brock 1995, Lescureux and Linnell 2014, Yilmaz 2012, Zeder 2008). The dog (*Canis familiaris*), which was domesticated from the wolf in the pre-agricultural age (Turnbull and Reed 1974), has been well characterized as containing more than 400 breeds with various morphological and behavioral traits. Still, there have been few studies on the genetic backgrounds and genetic polymorphisms between dogs' breeds generally and Pishdar breed in particular (Fredholm and Winterø 1995, Koskinen and Bredbacka 2000, Zajc et al. 1997).

The random amplification polymorphism DNA (RAPD) marker has been widely used and the first scientist who described the random amplified polymorphic DNA (RAPD) was Welsh and Mcclelland (1990) and Williams et al. (1990). The main advantage of this technique that is being easy to use, quick and consider as much influenced way for producing species-specific fingerprints. The method employed short oligonucleotide primers of arbitrary sequence to amplify unknown fragments of genomic DNA (Stêpniak et al. 2002). RAPD technique does not enquiry any previous knowledge to preforming the reaction of genome under investigation.

And the result of this technique depends on detected as a band's presence or absence, RAPD has been applied for population analysis in detecting genetic diversity in humans, insect, bacteria, plants, birds and in estimation breeding in cattle, sheep and goat, and recently in dogs, few investigation of using RAPD

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Table 1. Primers name, sequence and GC % for all primers used

No.	Primer name	Sequence (5-3')	GC contents %
1	OPA-04	AATCGGGCTG	60
2	OPA-14	TCTGTGCTGG	60
3	OPA-20	GTTGCGATCC	60
4	OPB-01	GTTTCGCTCC	60
5	OPB-07	GGTGACGCAG	70
6	OPB-12	CCTTGACGCA	60
7	OPB-19	ACCCCCGAAG	70
8	OPC-02	GTGAGGCGTC	70
9	OPC-13	AAGCCTCGTC	60
10	OPG-03	GAGCCCTCCA	70
11	OPG-05	CTGAGACGGA	60
12	OPM-05	GGGAACGTGT	60
13	OPM-06	CTGGGCAACT	60
14	OPM-20	AGGTCTTGGG	60
15	OPN-16	AAGCGACCTG	60
16	OPP-04	GTGTCTCAGG	60
17	OPQ-03	GGTCACCTCA	60
18	OPQ-07	CCCCGATGGT	70
19	OPS-01	CTACTGCGCT	60
20	10 MER	AACGCGCAAC	60

analysis in dogs genetic study (Gu et al. 1997). However, no studies based on the RAPD technique have been carried out in phylogenetic context relationship among the hosting regions for Pishdar dog.

Moreover, the goal of this study was to estimate phylogenetic relationships between the nine different hosting regions of Pishdar dog in Kurdistan Region / Iraq using RAPD-DNA marker technique.

MATERIALS AND METHODS

This study was managed on the Pishdar dog (nine hosting regions: Dokan, Suliamani, Sangasar, Huwana, Sitak, Halabja, Rania, Qala-Diza and Pishdar). Blood samples were collected at different regions in Suliamani governorate. A total of 74 dogs were sampled. Five mL of whole blood was collected from each animal from jugular vein into 10 mL vacutainer tubes containing the anticoagulant, ethylenediaminetetra-acetic acid (EDTA) and blood samples were stored at -20 ˚C until DNA extractions. DNA is extract from each of the blood sample using DNA Blood Mini Kit (Geneaid, Korea), and plus Quick-gDNA Miniprep kit (Zymo Company, USA). The DNA samples for each region mixed together to make one sample (pooled sample). The quantity and quality of DNA were determined by Nanodrop spectrophotometer and 1% agarose gel electrophoresis, respectively. In the present study, fourteen RAPD primers which were obtained from CinnaGen Inc.; (Iran) were used. The descriptions of primers regarding their names, primer sequences, GC percentages are given in **Table 1**.

RAPD technique were performed as described by Williams et al. (1990). RAPD PCR product was complete in a volume of 20 µl containing: 50 ng of genomic DNA, Concentration 2 µl volume of arbitrary primer, 200 mM dNTPs, 1.5 mM MgCl2, 1 unit of Taq. Negative controls (lacking DNA) were set up for each reaction master mix to check for DNA contamination. The Polymerase Chain Reaction (PCR) program used for Primer (OPQ-03): programmed for 35 cycles of denaturation at 95°C for 0.15 minutes (min), annealing at 32°C for 0.30 min and extension at 72°C for 1 min. An initial denaturation step of 5 min at 95°C and a final extension step of 7 min at 72°C were included in the first and last cycles, respectively. For the (OPA-14, OPG-5, OPA-20 and OPQ-07), used the above program with annealing temperature replaced to 35°C ,and for Primers (OPM-6 and OPM-20), annealing temperature was set at 36°C ,and 4th protocol for primers (OPM-5, OPP-4 and OPS-1), annealing temperature was 37°C .Meanwhile, the last fifth protocol for primers (OPA-4 and OPB-1, OPN-16 and 10 MER), annealing temperature was 41°C. The amplification PCR product were loaded onto horizontal agarose gel electrophoresis A 1 % agarose gels, (1gr of Agarose powder melting with 100 ml of 1X TBE buffer, (0.89 M Tris-OH, 0.89 M boric acid and 0.11 M EDTA) heated the mixture reached the boiling temperature in a microwave oven, then when the mixture cooled to under 55 C. 5 µl of Ethidium bromide insert to the mixture and poured in the Agarose tray (Sambrook et al., 1989). Moreover, electrophoresis at 85 V for 90 min. The results of electrophoresis were documented with the use of the UV transillumination. The RAPD bands were scored as present (1) or absent (0) in each pattern. All genetic parameters in current study were calculated by using GENEPOP software (version, 3.3) (Raymond and Rousset, 1995).

RESULTS AND DISCUSSION

The Total Fragment Number (TFN)

The TFN in Pishdar dog were 709 bands for all fourteen arbitrary primers (**Figs. 1**-**5**) regarding to the nine different regions, the highest value of bands numbers among the nine geographical regions for Pishdar dog was a share the Sangasar region and it granted 90 bands. While the lowest value is, 74 bands were record for Rania region. Moreover, the band size ranged between 100-1800 pb, the smallest band size was in OPQ-07 loci while the highest value was found in OPA-20 primer. A hassling result was recorded even by Stêpniak et al. (2002) and Atasoy et al. (2014). These results were lower than that recorded by Olivier et al. (1999) which gave (500-3000) bp (**Table 2**).

Table 2. Band numbers and bands size range (bp) among nine geographical regions for Pishdar dog

#	Primer names	Dokan		Qala-Diza		Sitak		Pishdar		Rania		Halabja		Huwana		Sulliamani		Sangasar		Over all	
		no. of band	Size range bp	no. of band	Size range bp	no. of band	Size range bp	no. of band	Size range bp	no. of band	Size range bp	no. of band	Size range bp	no. of band	Size range bp	no. of band	Size range bp	no. of band	Size range bp	Total no. of band	Size range bp
1	OPA-04	5	400-1400	5	400-1400	5	400-1400	6	400-1400	5	400-1400	5	400-1400	5	400-1400	6	400-1400	6	400-1400	48	400-1400
2	OPB-01	5	600-1500	3	600-1100	3	600-1100	5	600-1500	5	600-1500	1	600	6	600-1500	1	600	5	600-1500	34	600-1500
3	OPM-06	8	400-1500	3	500-700	7	400-1100	8	400-1500	7	500-1500	7	400-1500	3	500-700	7	400-1500	8	400-1500	58	400-1500
4	OPQ-03	4	500-800	3	400-800	2	700-800	5	250-1500	3	400-800	4	500-1500	3	500-800	4	400-1500	5	250-1500	33	250-1500
5	OPA-14	3	650-800	3	650-800	6	650-1100	3	650-800	2	700-800	3	650-800	3	650-800	2	650-800	7	500-1100	32	500-1100
6	OPM-20	4	370-850	6	370-1050	7	370-1450	0	-	1	450	4	370-700	4	450-700	2	450-700	0	-	28	370-1450
7	OPQ-07	11	100-1600	8	100-1400	11	100-1500	8	100-1400	11	100-1600	10	100-1600	12	100-1600	10	100-1600	12	100-1600	93	100-1600
8	OPS-1	6	400-1600	8	200-1600	7	200-1600	6	400-1600	7	200-1600	6	400-1600	7	200-1600	6	400-1600	6	400-1600	59	200-1600
9	OPM-05	2	1100-1500	2	1100-1500	2	1100-1500	2	1100-1500	0	#VALUE!	2	1100-1500	2	1100-1500	1	1100	2	1100-1500	15	1100-1500
10	OPP-04	2	700-1000	6	350-1500	6	350-1500	3	700-1500	4	400-900	5	350-1500	3	350-700	6	350-1500	6	350-1500	41	350-1500
11	OPN-16	6	200-900	9	200-1500	7	400-1100	10	200-1500	7	350-1400	10	200-1500	9	300-1500	10	200-1500	10	200-1500	78	200-1500
12	OPG-05	10	250-1700	9	250-1700	9	250-1700	8	250-1700	9	250-1700	9	250-1700	9	250-1700	9	250-1700	9	250-1700	81	250-1700
13	10-MER	8	300-1600	8	300-1600	8	300-1600	8	300-1600	8	300-1600	8	300-1600	9	300-2500	8	300-1600	8	300-1600	89	300-1600
14	OPA-20	6	300-1800	4	300-1500	8	400-1800	7	300-1800	5	300-1800	6	600-1800	6	600-1800	6	300-1800	6	600-1800	54	300-1800
	Total	80	100-1800	77	100-1700	88	100-1800	79	100-1800	74	100-1800	80	200-1800	81	100-2500	78	100-1800	90	100-1800	709	100-1800

Fig. 1. Gel electrophoresis for fourteen RAPD primers in studied Pishdar dog breed among nine different regions (Si=Sitak region, Hu=Huwana region, H=Halabja region, D=Dokan region, R=Rania region, Q=Qala-Diza region, Su= Suliamani region, Sa=Sangasar region, P=Pishdar region, C=Control sample)

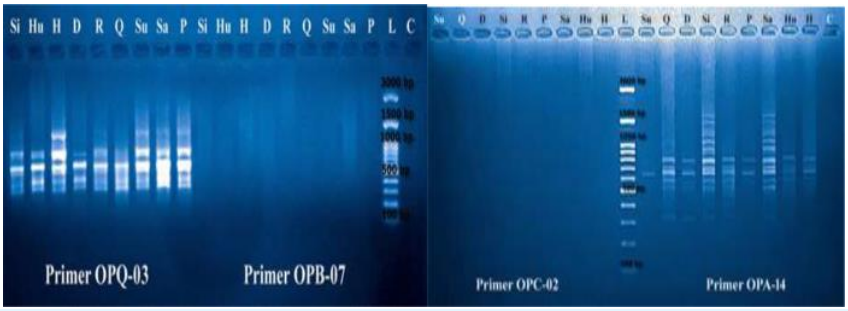

Fig. 2. Gel electrophoresis for fourteen RAPD primers in studied Pishdar dog breed among nine different regions (Si=Sitak region, Hu=Huwana region, H=Halabja region, D=Dokan region, R=Rania region, Q=Qala-Diza region, Su= Suliamani region, Sa=Sangasar region, P=Pishdar region, C=Control sample)

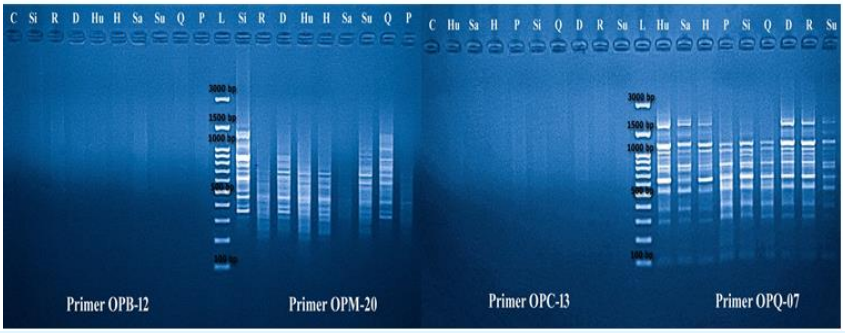

Fig. 3. Gel electrophoresis for fourteen RAPD primers in studied Pishdar dog breed among nine different regions (Si=Sitak region, Hu=Huwana region, H=Halabja region, D=Dokan region, R=Rania region, Q=Qala-Diza region, Su= Suliamani region, Sa=Sangasar region, P=Pishdar region, C=Control sample)

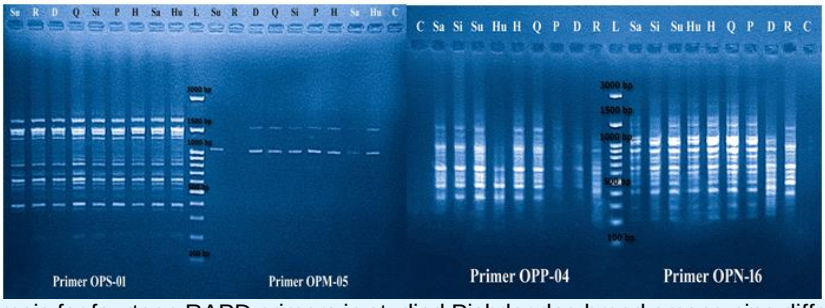

Fig. 4. Gel electrophoresis for fourteen RAPD primers in studied Pishdar dog breed among nine different regions (Si=Sitak region, Hu=Huwana region, H=Halabja region, D=Dokan region, R=Rania region, Q=Qala-Diza region, Su= Suliamani region, Sa=Sangasar region, P=Pishdar region, C=Control sample)

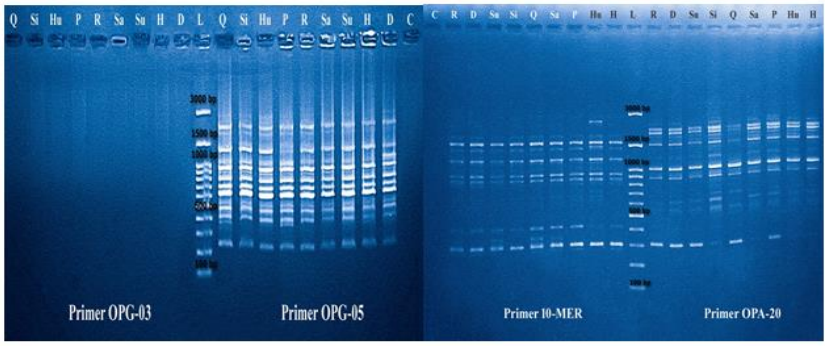

Fig. 5. Gel electrophoresis for fourteen RAPD primers in studied Pishdar dog breed among nine different regions (Si=Sitak region, Hu=Huwana region, H=Halabja region, D=Dokan region, R=Rania region, Q=Qala-Diza region, Su= Suliamani region, Sa=Sangasar region, P=Pishdar region, C=Control sample)

Table 3. Overall NPF and PPF for all primers used

No.	Primer name	No. of polymorphic bands	Polymorphic bands (%)
1	OPA-04	1	14.28
2	OPB-01	4	66.66
3	OPM-06	6	75
4	OPQ-03	4	57.14
5	OPA-14	5	71.42
6	OPM-20	8	80
7	OPQ-07	6	50
8	OPS-1	1	12.5
9	OPM-05	2	100
10	OPP-04	5	55.55
11	OPN-16	8	61.53
12	OPG-05	1	10
13	10-MER	0	0
14	OPA-20	6	66.66
Mean		4.07	51.48

Number of Polymorphic Fragments (NPF) and Percentage of Polymorphic Fragments (PPF)

By the selection of the fourteen primers, 709 bands were found, only 57 out of them were polymorphic in location groups of Pishdar dog. However, the overall mean of (NPF) was 4.07 with the highest value found at loci OPM-20 and OPN-16 scored eight bands. Over that, the smallest average of polymorphic bands number was found in (10-MER, OPA-04, OPS-01, OPG-05 and OPM-05) with average between (0-2) NPF. Meanwhile the (OPB-01, OPQ-03 and OPP-04) loci recorded more than three NPF. As well as, the overall mean PPB for the fourteen primers in the current study was 51.48%. As in the outcomes, only one primer have 100% PPF in (OPM-05), and the lowest polymorphism were detected in four primers (10-MER, OPG-05, OPS-01 and OPA-04) and gave (0%, 10%, 12.5% and 14.28%) PPF, respectively. These results specified the opportunity of depending on these loci for genetic characterization or genetic distances among the present Pishdar dog. The NPF in present study was higher than that reported by Rothuizen and Van Wolferen (1994) in the six groups of beagles. Furthermore, the NPF in present data was lower than that assayed by Olivier et al. (1999). From twelve DNA samples pedigree of Labrador Retrievers breed. As shown in **Table 3**.

Gene Frequency

The mean of gene frequency for all loci for allele 0 was (0.32) reached from 0.11 for OPM-06, locus, and 0.44 for OPB-01, OPQ-03, OPM-20, OPA-20, loci. And allele 1 got (0.66), ranged from 0.55 for the primers (OPB-01, OPQ-03, OPM-20 and OPA-20) and 0.88 for primer OPM-06. This result was higher than that reported by Altet et al. (2001) in a pool of 95 dogs token from 24 different breeds, and also higher than the Golden retriever breeds, but lower than that recorded in Labrador retriever and Rottweilers breeds. Furthermore, the result show gene frequency more than that noticed in Kangal and Akbash breed were they records average of allele frequency (0.41,0.37) respectively (Ajani et al. 2015, Erdoğan and Özbeyaz 2004).

Table 4. Overall gene frequency for all primers used

No.	Locus	Allele 0*	Allele 1**
1	OPA-04	0.33	0.66
2	OPB-01	0.44	0.55
3	OPM-06	0.11	0.88
4	OPQ-03	0.44	0.55
5	OPA-14	0.22	0.77
6	OPM-20	0.44	0.55
7	OPQ-07	0.33	0.66
8	OPS-1	0.33	0.66
9	OPM-05	0.22	0.77
10	OPP-04	0.33	0.66
11	OPN-16	0.33	0.66
12	OPG-05	0.22	0.77
13	10-MER	0.33	0.66
14	OPA-20	0.44	0.55

* Mean the absent of the band; ** Mean the present of the band

Unique Bands/Fragments

Fifteen unique bands out of 709 polymorphic bands were recorded for the regional group in the Pishdar dog. The highest unique band was found in the Rania region that scored five unique bands out of fifteen unique bands an ranged in size (400-1400) bp. Meanwhile, Dokan, Huwana and Sitak gave (2-3) unique bands. Besides, one unique band was seen in the Qala-Diza, Suliamani and Sangasar regions. Hence, Rania region shows more genetic distance from other geographical regions suggest that the individuals in this region might be exposed to several factors such as, selection, genetic drift, mutation and migration. This finding was in congruent with (Atasoy et al. 2014) in Malaklı Karabaş dog, and (Erdoğan et al. 2013), as seen in **Table 5**.

Nei's Gene Diversity (H) and Shannon's Information Index (I) for all loci

As in **Table 6**, the means of heterozygosity ratio for all fourteen primers in region group of the Pishdar dog averaged 0.41, the highest heterozygosis value were seen in (OPB-01, OPQ-03, OPM-20 and OPA-20) with average (0.49). While the primer OPM-06 recorded the lowest (H) ratio (0.19). Furthermore, the average Shannon diversity index value was (0.60) ranging from 0.34 to 0.68. Here, the result was lower than that seen in Koskinen and Bredbacka (2000), Ichikawa et al. (2001), Larson et al. (2012) and Atasoy et al. (2014) and similar to that recorde by Erdoğan et al. (2013) where is the average heterozygosity values for Akbash and Kangal are 0.367 and 0.410, respectively.

Table 6. Observed number, effective number of allele, gene diversity and Shannon information index for all loci

No.	locus	Sample size	Na*	Ne*	H*	I*
1	OPA-04	9	2	1.8	0.44	0.63
2	OPB-01	9	2	1.97	0.49	0.68
3	OPM-06	9	2	1.24	0.19	0.34
4	OPQ-03	9	2	1.97	0.49	0.68
5	OPA-14	9	2	1.52	0.34	0.52
6	OPM-20	9	2	1.97	0.49	0.68
7	OPQ-07	9	2	1.8	0.44	0.63
8	OPS-1	9	2	1.8	0.44	0.63
9	OPM-05	9	2	1.52	0.34	0.52
10	OPP-04	9	2	1.8	0.44	0.63
11	OPN-16	9	2	1.8	0.44	0.63
12	OPG-05	9	2	1.52	0.34	0.52
13	10-MER	9	2	1.8	0.44	0.63
14	OPA-20	9	2	1.97	0.49	0.68
Mean			2	1.75	0.41	0.6
SD±			0	0.21	0.08	0.09

*Na=observation numbers of alleles, Ne=effective numbers of alleles, H=Nei's (1973) gene diversity,I=Shannon's information index (Lewontin, 1972), SD± standard deviation.

Since the assay, the result poorly characterized low heterozygosity value and high Shannon index diversity. These problems can overcome suggestion of being this population has a similar genetic structure and the breeding strategy has been optional or the inbreeding values was be higher in this breed of dog.

Genetic Distance and Phylogenetic Tree among Dogs in Different Region

The Nei's genetic distance was ranged from 7.70 to 56.13, and the highest value of genetic distance found between (Sitak and Huwana) in addition to find between

Table 5. Unique band numbers and fragments size among dogs in the geographical regions

#	Primer names	Dokan		Qala-Diza		Sitak		Pishdar		Rania		Halabja		Huwana		Suliamani		Sangasar		Overall	
		No. of unique. band	Size range bp Fragments size (bp)	No. of unique band	Size range bp Fragments size (bp)	no. of unique band	Size range bp Fragments size (bp)	no. of unique. band	Size range bp Fragments size (bp)	no. of unique. band	Size range bp Fragments size (bp)	no. of unique. band	Size range bpFragments size (bp)	no. of unique. band	Size range bpFragments size (bp)	no. of unique. band	Size range bpFragments size (bp)	no. of unique. band	Size range bpFragments size (bp)	Total No. of unique band	Fragment size range (bp)
1	OPA-04	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	700	0	-	1	700
2	OPB-01	0	-	0	-	0	-	0	-	0	-	0	-	1	700	0	-	0	-	1	700
3	OPM-06	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
4	OPQ-03	1	720	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	720
5	OPA-14	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	500	1	500
6	OPM-20	0	-	0	-	2	500-1450	0	-	0	-	0	-	0	-	0	-	0	-	2	500-1450
7	OPQ-07	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
8	OPS-1	0	-	1	700	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	700
9	OPM-05	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
10	OPP-04	0	-	0	-	0	-	0	-	3	400,600,900	0	-	0	-	0	-	0	-	3	400-900
11	OPN-16	0	-	0	-	0	-	0	-	2	450-1400	0	-	0	-	0	-	0	-	2	450-1400
12	OPG-05	1	1100	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	1100
13	10-MER	0	-	0	-	0	-	0	-	0	-	0	-	1	2500	0	-	0	-	1	2500
14	OPA-20	0	-	0	-	1	400	0	-	0	-	0	-	0	-	0	-	0	-	1	400
	Total	2	720-1100	1	700	3	400-500-1450	0	-	5	400,450,600,900,1400	0	-	2	700-2500	1	700	1	500	15	400-2500

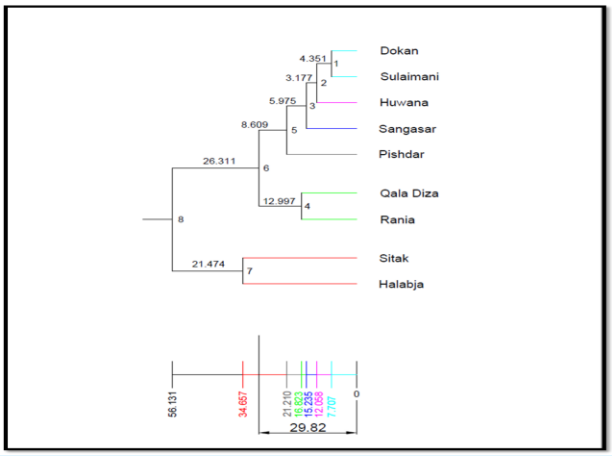

Fig. 1. UPGMA dendrograms showing differentiation among the fourteen locations based on Nei's (1972) genetic distance

(Huwana and Halabja) with an average 34.65 %. Moreover, the lowest genetic distance that documented was between (Dokan and Suliamani) and was averaged (7.70). Phylogenetic tree screen three main clumps were created, the first clumps tree was between two population (Sitak and Halabja) and recorded (34.657%). The second main cluster involved (Qala-Diza and Rania) and with a genetic range (16.82%).

However, the third clumps tree constitutes four sub-clumps the first branch consists of (Dokan and Suliamani) region, the second branch harbored the Huwana region only. The third one covers the Sangasar region. Finally, the fourth sub-cluster possesses the Pishdar group. The genetic distance for these four sub-clusters were (7.707%, 12.058%, 15.235%, and 21.21%). The overall diversity arrived at 56.13 % , the study determine an impressive logical result, showed a low genetic distance between Dokan and Suliamani population, in addition to small genetic distance between Qala-Diza and Rania, and moderate genetic distance between Sitak and Halabja.which means there was no genetic variation in between these populations according to the near geographical distance between these areas. Thus the inbreeding mating among these areas records high value. Meanwhile, the Huwana, Sangasar and Pishdar sub-clusters population documented a moderate genetic distance between them. Nevertheless, the high genetic distance that recorded (56.13%) among the region's population of Pishdar dog showed ample ground for mating within this breed in suliamani province. these results were similar and /or slightly higher than that checked by Erdoğan and Özbeyaz (2004), and higher genetic distance seen in Malaklı Karabas dogs among ten regions (Atasoy et al., 2014). The rate in homozygote in all populations is higher than that expected in the Hardy-Weinberg balance.This means that there is not random mating in populations or that there is inbreeding, besides the result shows that the populations have similar genetic structures among the location groups (**Fig. 1**).

CONCLUSION

The data acquired from the lines included in this study are of great importance to the Pishdar dog owners in Kurdistan region - Iraq. The distance (7.707 to 56.13) and polymorphism (14 to 80%) among nine geographic areas established in this study. As well as results of this study help us to clarify the image of the genetic Diversification of the local Iraqi Pishdar dog breeds in Suliamani province and the dogs' owners can use it for the Copulating system when required to make cross dog breed. Eventually, to have the precise evaluation of the genetic distance of these local genetic resources.

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