Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Phylogenetic analysis based on ITS1 gene of *Leishmania* lineage: Meta-analysis using in-silico techniques

Leishmania soyunun ITS1 genine dayalı filogenetik analiz: In-silico teknikleri kullanılarak meta-analiz

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ABSTRACT

Objective: Leishmaniasis is a parasitic disease caused by more than 20 *Leishmania* species. This disease is spread by vectors. Many researchers agree that *Leishmania* was spread to mammals by sandflies of the genus *Phlebotomus* and *Lutzomyia*. Leishmaniasis is still considered one of the most neglected diseases by the World Health Organization (WHO). An estimated 0.7-1 million new cases of leishmaniasis are reported annually from approximately 100 endemic countries. The types of leishmaniasis in humans are the visceral (VL), cutaneous (CL), mucocutaneous (MCL), diffuse cutaneous (DCL), and post kala-azar dermal (PKDL) forms of Leishmaniasis. The aim of this study is to perform phylogenetic analysis of *Leishmania* origin based on ITS1 gene region using in-silico techniques. In this way, it is also aimed to take a snapshot of a meta-analysis of vertical and horizontal spread at the global level.

Methods: In this study, *Leishmania* ITS1 region sequences presented with the GenBank data of the National Center for Biotechnology Information, USA, (NCBI) until 15.05.2019 were taken and analyzed by in-silico techniques. 914 sequences were obtained for the *Leishmania* ITS1 region in the NCBI database. All

ÖZET

Amaç: Leishmaniasis, 20'den fazla *Leishmania* türü tarafından oluşturulan paraziter bir hastalıktır. Bu hastalık vektörler tarafından yayılmaktadır. Birçok araştırmacı, *Leishmania*'nın memelilere *Phlebotomus* ve *Lutzomyia* cinsi tatarcık sinekleri tarafından yayıldığı konusunda hemfikirdir. Leishmaniasis, halen Dünya Sağlık Örgütü (WHO) tarafından en çok ihmal edilen hastalıklardan biri olarak kabul edilmektedir. Yaklaşık 100 endemik ülkeden yılda tahmini 0.7-1 milyon yeni leishmaniasis vakası bildirilmektedir. İnsanlardaki leishmaniasis türleri, Leishmaniasis'in visseral (VL), kutanöz (CL), mukokutanöz (MCL), diffüz kutanöz (DCL) ve post kala-azar dermal (PKDL) formlarıdır. Bu çalışmanın amacı, in-silico teknikler kullanılarak *Leishmania* kökeninin ITS1 gen bölgesine dayalı filogenetik analizini gerçekleştirmektir. Bu yolla, küresel düzeyde vertikal ve horizontal yayılımın meta-analizi ile anlık bir görüntü almak da amaçlanmıştır.

Yöntem: Bu çalışma ile 15.05.2019 tarihine kadar National Center for Biotechnology Information, USA, (NCBI) GenBank verileri ile sunulan *Leishmania* ITS1 bölge sekansları alınarak in-silico tekniklerle analiz edilmiştir. NCBI veritabanında *Leishmania* ITS1 bölgesi

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Geliş Tarihi / Received : 15.02.2023
Kabul Tarihi / Accepted : 22.09.2023

DOI ID : 10.5505/TurkHijyen.2023.90083

Güldemir D, Yücesan B. Phylogenetic analysis based on ITS1 gene of *Leishmania* lineage: Meta-analysis using in-silico techniques.
Turk Hij Den Biyol Derg, 2023; 80(4): 429 - 444

sequences were examined and sequences without indel problems were selected from these strains mapped according to the consensus sequence. It was decided to form a phylogenetic tree with the forms that were examined and 65 strains were obtained by removing the sub-branches.

Results: The phylogenetic tree obtained in this study showed that *Leishmania* strains clustered in six branches according to the ITS1 region. Here, a phylogenetic tree is drawn and the molecular epidemiological and demographic data of these six generations and beyond, which are obtained as a result of the genetic relationships between the strains, are summarized.

Conclusion: In conclusion, Leishmaniasis is an important public health problem that can be seen in many developing countries. In this study, the strains examined using the in-silico method were isolated from different geographies of the world between 1984 and 2018. The phylogenetic relationships between these strains show not only the vertical spread of the origins over the years, but also the horizontal spread geographically. These species were obtained from different host and tissue types. Thus, the relationships of *Leishmania* strains in the host-vector-reservoir chain are explained. Therefore, it is clear that there is a need for more meta-analysis studies such as this study on factors and their diffusion.

Key Words: *Leishmania*, ITS1 gene, in silico meta-analysis, phylogenetic study

için 914 dizi elde edildi. Tüm sekanslar incelenmiş ve konsensüs sekansına göre haritalanan bu suşlardan ekle-sil (indel) problemi olmayan sekanslar seçilmiştir. İncelenen ve alt dalları çıkarılarak 65 suş elde edilen formlar ile filogenetik ağacın oluşturulmasına karar verilmiştir.

Bulgular: Bu çalışmada elde edilen filogenetik ağaç, *Leishmania* suşlarının ITS1 bölgesine göre altı kolda kümelendiğini göstermiştir. Burada filogenetik bir ağaç çizilerek suşlar arasındaki genetik ilişkiler sonucunda elde edilen bu altı kuşak ve ötesine ait moleküler epidemiyolojik ve demografik veriler özetlenmiştir.

Sonuç: Sonuç olarak Leishmaniasis, gelişmekte olan birçok ülkede görülebilen önemli bir halk sağlığı sorunudur. Bu çalışmada in-silico yöntemi kullanılarak incelenen suşlar 1984-2018 yılları arasında dünyanın farklı coğrafyalarından izole edilmiştir. Bu suşlar arasındaki filogenetik ilişkiler, kökenlerin yıllara göre sadece dikey yayılımını değil, aynı zamanda coğrafi olarak yatay yayılımı da göstermektedir. Bu türler farklı konak ve doku tiplerinden elde edilmiştir. Böylece *Leishmania* suşlarının konak-vektör-rezervuar zincirindeki ilişkileri açıklanmaktadır. Bu nedenle, faktörler ve bunların yayılımı üzerine bu çalışma gibi çok sayıda meta-analiz çalışmasına ihtiyaç olduğu açıktır.

Anahtar Kelimeler: *Leishmania*, ITS1 geni, in silico meta-analiz, filogenetik analiz

INTRODUCTION

Leishmaniasis is an important public health problem that can be seen in many developing countries (1). However, the emergence of unexpected *Leishmania* species in unexpected areas beyond our classical knowledge due to population travel and migration (2). Classically, human dog rodents are reservoirs and sandflies are vectors (3). Leishmaniasis ranges from localized cutaneous (CL), mucocutaneous (MCL), and diffuse visceral leishmaniasis (VL), which can be fatal (4,5).

In recent years, molecular techniques in the diagnosis and typing of Leishmaniasis are rapidly developing and their use is becoming widespread worldwide. Especially with increasing genome studies, *Leishmania* taxonomy was reconstructed and it was shown that the *Leishmania* genus was shown to be separated into *Viannia* and *Leishmania* subgenuses approximately 20-100 million years ago with ancestral DNA studies (6). DNA sequence analysis of some gene regions was used to evaluate phylogenetic relationships in *Leishmania* lineage such as ITS1 and ITS2 regions, Mini-exon/Spliced Leader (rDNA), *gp63*, *hsp70*, cpb, POLA, G6PD, 6PGDH, MPI, Histones, RPOIILS, NAGT, A2, EF-1α (proein coding gene), *cytb*, *COII* (kDNA maxicircle) and kDNA minicircle. The most used among them is the sequence of the ITS1 region (7) ITS1 is a non-coding region placed at SSUrRNA, which is bounded by the genes 18S and 5.8S that produce a 300-350 bp fragment of *Leishmania* spp. (7). In this study, ITS1 region was selected for phylogenetic analysis of current *Leishmania* lineage. As it is known, in silico studies enable the evaluation of large-scale genomic data with the innovations offered by information technologies, bioinformatics and artificial intelligence tools, and are becoming increasingly widespread. The aim of this study is to snapshot a meta-analysis of vertical and horizontal propagation at the global level by performing phylogenetic analysis based on *Leishmania* lineage ITS1 gene using in-silico techniques.

MATERIAL and METHOD

In our study, *Leishmania* ITS1 region sequences submitted to National Center for Biotechnology Information, USA, (NCBI) GenBank (www.ncbi.nlm.nih.gov) until 15.05.2019 were analyzed by in-silico techniques (8).

In the NCBI database, 914 sequences were obtained for the *Leishmania* ITS1 region. These data were uploaded to the Geneious 11.0.5. (www.geneious.com) platform in FASTA format (9). Genome mapping was performed on these sequences using the *Leishmania* GQ333260.1 strain. All strains mapped according to the consensus sequence were examined and those without indel problems were selected. Approximately 350 bp lenght sequences covering the ITS1 region were extracted among them. As a result, 151 sequences were obtained. These DNA sequences were trimmed at both ends and their lengths were equalized. Then phylogenetic tree was drawn with these sequences.

The phylogenetic tree was created using the Geneious tree tool available on the Geneious 11.0.5 platform (www.geneious.com). It was examined, and decided to draw the phylogenetic tree with 65 strains by removing the sub-branches (Figure 1,2). Herein, a naming was made for the strains consisting of accession number, species, location and year data such as KF899857.1 (Lm/Iran/2013). The strain names given here are used in the phylogenetic tree (Figure 2). In addition, using the Create Maps: Scribble Maps (https://www.scribblemaps.com/create/) application, the regions where the strains in this study are isolated are marked on the world map and the geographical spreads of the clades are shown Figure 3 (10).

RESULTS

In this study, the strains examined using the in-silico method were isolated from different geographies of the world between 1984-2018. The phylogenetic relationships between these strains show not only the vertical spread of origins over the years, but also the horizontal spread as geographically.

The phylogenetic tree obtained in our study shows that *Leishmania* strains were clustered in six clades (Figure 2). The vertical, horizontal and interspecies spread of the selected for the present study *Leishmania* species according to the ITS1 gene region is summarized in Table 1. The demographic characteristics of the strains allocated to the *Leishmania* ITS1 region for genogroups, along with their names and related publications are given table 2. In addition, the regions where the strains in this study are isolated are geographically marked on the world map and the geographical spread of clades shown as Figure 3.

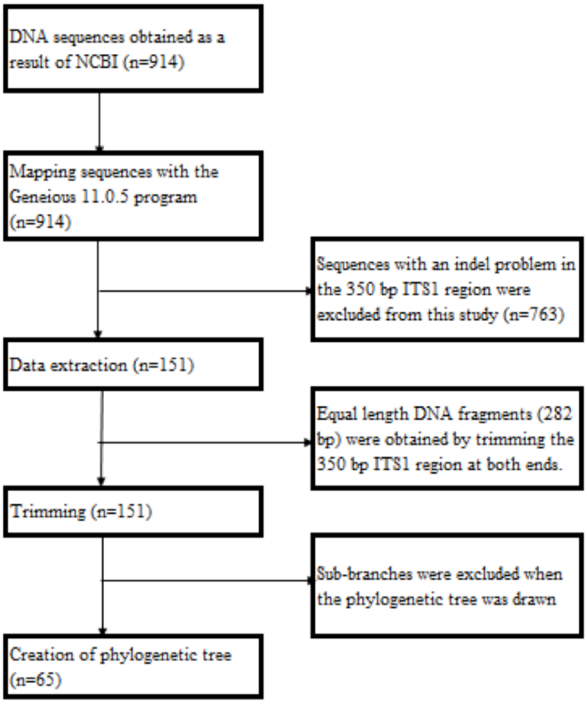

Figure 1. Work flow chart for deciding *Leishmania* ITS1 sequences to take place in this study

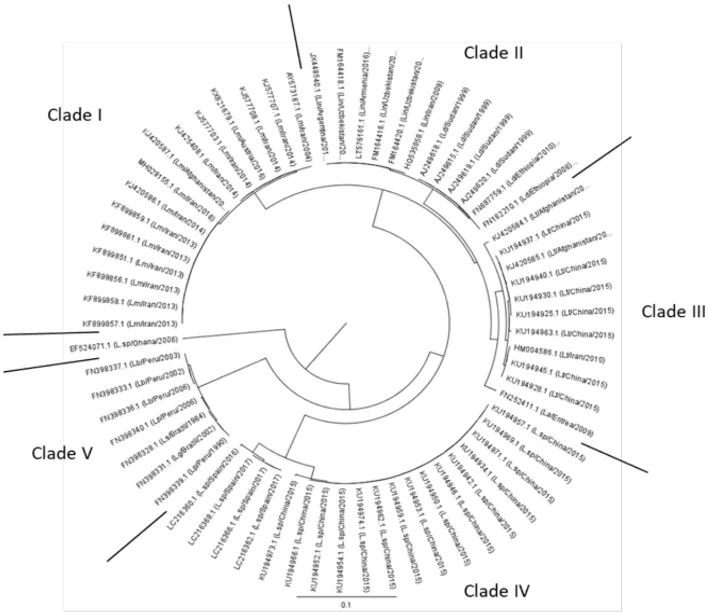

Figure 2. The phylogenetic tree obtained in this study shows that *Leishmania* strains (n=65) were clustered in six clades. The figure shows that Clade I (n = 2), Clade II (n = 3), Clade III (n = 5), Clade IV (n = 4), Clade V (n = 3) subbranches (n: Number of sub-branches). The phylogenetic tree was created using the Geneious tree tool available on the Geneious 11.0.5 platform (www.geneious.com)

Figure 3. Global distribution of *Leishmania* genogroups analyzed in this study. Yellow: Clade I, Green: Clade II, Pink: Clade III, Turquoise: Clade IV, Blue: Clade V: White: EF524071.1 (This strain is genotypically remote and unique from other clades in present study). Create Maps: Scribble Maps (https://www.scribblemaps.com/create/) application was used in map production

Table 1. Vertical, horizontal and inter-species spread of *Leishmania* species according to ITS1 gene region

	Leishmania spp.	Vertical Propagation (Years)	Horizontal Propagation (Geographic)	Inter-species Spread
Clade I	*L. major*	2004-2018	Iran, Afghanistan, Austria	*Rhombomys opimus* (Rodent)- *Phlebotomus papatasi* (Sand fly) - *Homo sapiens* (Human)
Clade II	*L. infantum,* *L. donovani*	1999-2016	Iran, Uzbekistan, Armenia, Sudan, Ethiopia, Argentina	*Canis familiaris* (Dog) *P. tobbi* (Sand fly) *H. sapiens* (Human)
Clade III	*L. tropica,* *L. aethiopica*	2009-2015	Afghanistan, China, Iran Eritrea	*Eremias vermiculata,* *E. velox rborowskii,* *Phrynocephalus axillaris* (Desert lizards) *Canis spp.*(Dog) *H. sapiens* (Human)
Clade IV	*Leishmania* sp. (Not compatible with known species; high-similarity with *L. tarantolae* and *L. adleri)*	2015-2017	China, Spain	*E. vermiculata,* *E. velox rborowskii,* *E. multiocellata,* *P. axillaris,* *Tenuidactylus elongatus* (Desert lizards) *Sergentomyia minuta* (Sand fly)
Clade V	*L. (V.) braziliensis,* *L. (V.) shawi,* *L. (V.) guyanensis,* *L. (V.) peruviana*	1984-2006	Brazil, Peru	*Cebus apella* (monkey)- *H. sapiens* (Human)
Clade VI	*Leishmania sp.* (Not compatible with known species)	2006	Ghana	*H. sapiens* (Human)

Table 2. The demographic characteristics of the strains allocated to the Leishmania ITS1 region for genogroups, along with their names and related publications

No	Accession No	Name¹⁾	Country	Region	Isolation date	Host	Submitters (From NCBI)	Clade	Publications	Related References
1	KF899857.1	KF899857.1 (Lm/Iran/2013)	Iran	Ilam province, Dasht-e-Abbas	2013	Homo sapiens=”skin lesion	Karimian et al. 2013	I	Unpublished	11-18, 30
2	KF899858.1	KF899858.1 (Lm/Iran/2013)	Iran	Ilam province, Dasht-e-Abbas	2013	Homo sapiens=”skin lesion	Karimian et al. 2013	I	Unpublished	
3	KF899856.1	KF899856.1 (Lm/Iran/2013)	Iran	Ilam province, Dasht-e-Abbas	2013	Homo sapiens=”skin lesion	Maleki Ravasan et al. 2013	I	Unpublished	
4	KF899851.1	KF899851.1 (Lm/Iran/2013)	Iran	Ilam province, Dasht-e-Abbas	2013	Homo sapiens=”skin lesion	Maleki Ravasan et al. 2013	I	Unpublished	
5	KF899861.1	KF899861.1 (Lm/Iran/2013)	Iran	Ilam province, Dehloran	2013	Homo sapiens	Karimian et al. 2013	I	Unpublished	
6	KF899859.1	KF899859.1 (Lm/Iran/2013)	Iran	Ilam province, Mehran	2013	Homo sapiens =”skin lesion”	Karimian et al. 2013	I	Unpublished	
7	MH029155.1	MH029155.1 (Lm/Iran/2018)	Iran	Bam county,Khvajeh Askar	2018	Phlebotomus papatasi	Amiri Ghanat Saman et al. 2018	I	Unpublished	
8	KJ420586.1	KJ420586.1 (Lm/Iran/2014)	Iran	Ilam province, Mehran	2014	Homo sapiens=”skin lesion”	Mosawi et al. 2014	I	Mosawi and Dalimi 2015	
9	KJ420587.1	KJ420587.1 (Lm/Afghanistan/2014)	Afghanistan	Herat	2014	Homo sapiens=”skin lesion”	Mosawi et al. 2014	I	Mosawi and Dalimi 2015	
10	KJ425408.1	KJ425408.1 (Lm/Iran/2014)	Iran	North-East of Iran	2014	Phlebotomus papatasi	Rassi et al. 2014	I	Rafizadeh et al. 2016	
11	KJ577703.1	KJ577703.1 (Lm/Iran/2014)	Iran	Esfarayen district	2014	Rhombomys opimus	Rassi et al. 2014	I	Unpublished	
12	KJ577707.1	KJ577707.1 (Lm/Iran/2014)	Iran	Esfarayen district	2014	Rhombomys opimus	Rassi et al. 2014	I	Unpublished	
13	KJ577708.1	KJ577708.1 (Lm/Iran/2014)	Iran	Esfarayen district	2014	Rhombomys opimus	Rassi et al. 2014	I	Unpublished	
14	AY573187.1	AY573187.1 (Lm/Iran/2004)	Iran		2004	Homo sapiens	Tashakori et al. 2004	I	Unpublished	
15	KX821679.1	KX821679.1 (Lm/Austria/2016)	Austria		2016	Homo sapiens	Harrison et al. 2016	I	Harrison et al. 2017	

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No	Accession No	Name$^{1)}$	Country	Region	Isolation date	Host	Submitters (From NCBI)	Clade	Publications	Related References
16	HQ535858.1	HQ535858.1 (Lin/ Iran/2009)	Iran	northwestern Iran	2009	Phlebotomus tobbi=”sand fly”	Oshaghi et al. 2010	II	Rassi et al. 2012	19-25
17	FM164418.1	FM164418.1 (Lin/ Uzbekistan/2007)	Uzbekistan		2007	Homo sapiens	Alam et al. 2008	II	Alam et al. 2009	
18	FM164416.1	FM164416.1 (Lin/ Uzbekistan/2007)	Uzbekistan		2007		Alam et al. 2008	II	Alam et al. 2009	
19	FM164420.1	FM164420.1 (Lin/ Uzbekistan/2007)	Uzbekistan		2007		Alam et al. 2008	II	Alam et al. 2009	
20	LT576161.1	LT576161.1 (Lin/ Armenia/2016)	Armenia		2016		Kuhls et al. 2016	II	Sukiasyan et al. 2019	
21	JX448540.1	JX448540.1 (Lin/ Argentina/2012)	Argentina	Posadas, Misiones	2012	Canis familiaris=”epitelial cells from conjuntivas”	Acardi et al. 2012	II	Unpublished	
22	AJ249618.1	AJ249618.1 (Ld/ Sudan/1999)	Sudan	Eastern Sudan	1999		el Tai 1999	II	el Tai et al. 2000	
23	AJ249615.1	AJ249615.1 (Ld/ Sudan/1999)	Sudan	Eastern Sudan	1999		el Tai 1999	II	el Tai et al. 2000	
24	AJ249619.1	AJ249619.1 (Ld/ Sudan/1999)	Sudan	Eastern Sudan	1999		el Tai 1999	II	el Tai et al. 2000	
25	AJ249620.1	AJ249620.1 (Ld/ Sudan/1999)	Sudan	Eastern Sudan	1999		el Tai 1999	II	el Tai et al. 2000	
26	FN687759.1	FN687759.1 (Ld/ Ethiopia/2010)	Ethiopia		2010	Homo sapiens=”skin lesion	Gelanew 2010	II	Unpublished	
27	FN182210.1	FN182210.1 (Ld/ Ethiopia/2008)	Ethiopia		2008	Visceral leishmaniasis in Ethiopia	Gelanew 2009	II	Unpublished	

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No	Accession No	Name[1]	Country	Region	Isolation date	Host	Submitters (From NCBI)	Clade	Publications	Related References
28	KJ420584.1	KJ420584.1 (Lt/ Afghanistan/2014)	Afghanistan	Herat	2014		Mosawi et al. 2014	III	Mosawi and Dalimi 2015	26,28-32
29	KJ420585.1	KJ420585.1 (Lt/ Afghanistan/2014)	Afghanistan	Herat	2014	Homo sapiens=”skin lesion	Mosawi et al. 2014	III	Mosawi and Dalimi 2015	
30	KU194937.1	KU194937.1 (Lt/ China/2015)	China	Nanhu Town, Hami County, Xinjiang Uygur Autonomus Region	2015	Eremias vermiculata	Zhang et al. 2015	III	[2]Zhang et al. 2016	
31	KU194940.1	KU194940.1 (Lt/ China/2015)	China	Lukchun Town, Shanshan County, Xinjiang Uygur Autonomus Region	2015	Phrynocephalus axillaris	Zhang et al. 2015	III	Zhang et al. 2016	
32	KU194930.1	KU194930.1 (Lt/ China/2015)	China	Kumtag Desert, Tuokexun County, Xinjiang Uygur Autonomus Region	2015	Eremias vermiculata	Zhang et al. 2015	III	Zhang et al. 2016	
33	KU194925.1	KU194925.1 (Lt/ China/2015)	China	Kumtag Desert, Tuokexun County, Xinjiang	2015	Eremias vermiculata	Zhang et al. 2015	III	Zhang et al. 2016	
34	KU194963.1	KU194963.1 (Lt/ China/2015)	China	Erpu Town, Hami City, Xinjiang Uygur Autonomus Region	2015	Phrynocephalus axillaris	Zhang et al. 2015	III	Zhang et al. 2016	
35	KU194945.1	KU194945.1 (Lt/ China/2015)	China	Lukchun Town, Shanshan County, Xinjiang Uygur Autonomus Region	2015	Eremias velox rborowskii	Zhang et al. 2015	III	Zhang et al. 2016	
36	KU194928.1	KU194928.1 (Lt/ China/2015)	China	Kumtag Desert, Tuokexun County, Xinjiang Uygur Autonomus Region” Northwest China	2015	Eremias vermiculata	Zhang et al. 2015	III	Zhang et al. 2016	
37	HM004586.1	HM004586.1 (Lt/ Iran/2010)	Iran	Isfahan	2010	dog	Mahmoudzadeh-Niknam et al. 2010	III	Mahmoudzadeh-Niknam et al. 2011	
38	FN252411.1	FN252411.1 (La/ Eritrea/2009)	Eritrea		2009	Homo sapiens	Gelanew 2009	III	Zanger et al. 2016	

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No	Accession No	Name[1)]	Country	Region	Isolation date	Host	Submitters (From NCBI)	Clade	Publications	Related References
39	KU194957.1	KU194957.1 (L.sp/ China/2015)	China	Anxi Extreme Arid National Nature Reserve, Guazhou County, Gansu Province	2015	Eremias vermiculata	Zhang et al. 2015	IV	Zhang et al. 2016	27-29
40	KU194969.1	KU194969.1 (L.sp/ China/2015)	China	Dunhuang Yardong National Geopark, Gansu Province" Northwest China	2015	Eremias vermiculata	Zhang et al. 2015	IV	Zhang et al. 2016	
41	KU194971.1	KU194971.1 (L.sp/ China/2015)	China	Dunhuang Yardong National Geopark, Gansu Province	2015	Eremias vermiculata	Zhang et al. 2015	IV	Zhang et al. 2016	
42	KU194934.1	KU194934.1 (L.sp/ China/2015)	China	Nanhu Town, Hami County, Xinjiang Uygur Autonomus Region	2015	Eremias vermiculata	Zhang et al. 2015	IV	Zhang et al. 2016	
43	KU194942.1	KU194942.1 (L.sp/ China/2015)	China	Lukchun Town, Shanshan County, Xinjiang Uygur Autonomus Region	2015	Eremias velox rborowskii	Zhang et al. 2015	IV	Zhang et al. 2016	
44	KU194946.1	KU194946.1 (L.sp/ China/2015)	China	Lukchun Town, Shanshan County, Xinjiang Uygur Autonomus Region	2015	Eremias velox rborowskii	Zhang et al. 2015	IV	Zhang et al. 2016	
45	KU194950.1	KU194950.1 (L.sp/ China/2015)	China	Lukchun Town, Shanshan County, Xinjiang Uygur Autonomus Region	2015	Eremias velox rborowskii	Zhang et al. 2015	IV	Zhang et al. 2016	
46	KU194953.1	KU194953.1 (L.sp/ China/2015)	China	Anxi Extreme Arid National Nature Reserve, Guazhou County, Gansu Province	2015	Eremias multiocellata	Zhang et al. 2015	IV	Zhang et al. 2016	
47	KU194959.1	KU194959.1 (L.sp/ China/2015)	China	Erpu Town, Hami City, Xinjiang Uygur Autonomus Region" Northwest China	2015	Phrynocephalus axillaris	Zhang et al. 2015	IV	Zhang et al. 2016	
48	KU194962.1	KU194962.1 (L.sp/ China/2015)	China	Erpu Town, Hami City, Xinjiang Uygur Autonomus Region	2015	Phrynocephalus axillaris	Zhang et al. 2015	IV	Zhang et al. 2016	

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No	Accession No	Name¹⁾	Country	Region	Isolation date	Host	Submitters (From NCBI)	Clade	Publications	Related References
49	KU194974.1	KU194974.1 (L.sp/ China/2015)	China	Kumux Town, Tuokexun County, Xinjiang Uygur Autonomus Region	2015	Eremias velox rborowskii	Zhang et al. 2015	IV	Zhang et al. 2016	27-29
50	KU194954.1	KU194954.1 (L.sp/ China/2015)	China	Anxi Extreme Arid National Nature Reserve, Guazhou County, Gansu Province	2015	Eremias multiocellata	Zhang et al. 2015	IV	Zhang et al. 2016	
51	KU194952.1	KU194952.1 (L.sp/ China/2015)	China	Lukchun Town, Shanshan County, Xinjiang Uygur Autonomus Region" Northwest China	2015	Eremias velox rborowskii	Zhang et al. 2015	IV	Zhang et al. 2016	
52	KU194966.1	KU194966.1 (L.sp/ China/2015)	China	Erpu Town, Hami City, Xinjiang Uygur Autonomus Region	2015	Phrynocephalus axillaris	Zhang et al. 2015	IV	Zhang et al. 2016	
53	KU194973.1	KU194973.1 (L.sp/ China/2015)	China	Tuokexun County, Xinjiang Uygur Autonomus Region" Northwest China	2015	Tenuidactylus elongatus	Zhang et al. 2015	IV	Zhang et al. 2016	
54	LC216362.1	LC216362.1 (L.sp/ Spain/2017)	Spain	Madrid	2017	Sergentomyia minuta	Gonzalez et al. 2017	IV	Gonzalez et al. 2017	
55	LC216366.1	LC216366.1 (L.sp/ Spain/2017)	Spain	Madrid	July-2016	Sergentomyia minuta	Gonzalez et al. 2017	IV	Gonzalez et al. 2017	
56	LC216368.1	LC216368.1 (L.sp/ Spain/2016)	Spain	Madrid	August-2016	Sergentomyia minuta	Gonzalez et al. 2017	IV	Gonzalez et al. 2017	
57	LC216360.1	LC216360.1 (L.sp/ Spain/2016)	Spain	Madrid	July-2016	Sergentomyia minuta	Gonzalez et al. 2017	IV	Gonzalez et al. 2017	

Table 2. The demographic characteristics of the strains allocated to the Leishmania ITS1 region for genogroups, along with their names and related publications

No	Accession No	Name1)	Country	Region	Isolation date	Host	Submitters (From NCBI)	Clade	Publications	Related References
58	FN398328.1	FN398328.1 (Ls/ Brazil/1984)	Brazıl	Para	1984	Cebus apella	Kuhls 2009	V	Unpublished	33-35
59	FN398331.1	FN398331.1 (Lg/ Brazil/2002)	Brazıl	Acre	2002	Homo sapiens	Kuhls 2009	V	Unpublished	
60	FN398339.1	FN398339.1 (Lp/ Peru/1990)	Peru		1990	Homo sapiens	Kuhls 2009	V	Unpublished	
61	FN398340.1	FN398340.1 (Lp/ Peru/2006)	Peru		2006	Homo sapiens	Kuhls 2009	V	Unpublished	
62	FN398333.1	FN398333.1 (Lb/ Peru/2002)	Peru		2002	Homo sapiens	Kuhls 2009	V	Unpublished	
63	FN398337.1	FN398337.1 (Lb/ Peru/2003)	Peru		2003	Homo sapiens	Kuhls 2009	V	Unpublished	
64	FN398336.1	FN398336.1 (Lb/ Peru/2006)	Peru		2006	Homo sapiens	Kuhls 2009	V	Unpublished	
65	EF524071.1	EF524071.1 (L.sp/ Ghana/2006)	Ghana		2006	Homo sapiens	Villinski et al. 2007	VI	Villinski et al. 2008	36

Molecular epidemiological and demographic data on these six clades and beyond, which were obtained as a result of genetic relationships between strains are summarized in Table 2. Strains naming (name)1) was created for the strains consisting of accession number, species, location and year data such as KF899857.1 (Lm/Iran/2013). 2)This table contains 22 strains from the study of Zhang, J., et al., 2016. These 22 strains included in our study are phylogenetically in two groups (Clade III and Clade IV). Those in Clade III are located in the northern part of China (Uyghur), while those in Clade IV are located in the north and east of China. These study sequences have been deposited in GenBank under accession numbers KT990127-KT990210 and KU194923-KU194975

DISCUSSION

Molecular epidemiological and demographic data of these six classes obtained as a result of genetic relationships between strains by drawing a phylogenetic tree are summarized in Table 2. The literature for these strains is reviewed and the findings for molecular epidemiological relationships are discussed below.

In this study, the strains in Clade I (CI) originated from Iran, Afghanistan and Austria between 2004-2018, and all of are *Leishmania major*. The Austrian *L. major* strain (KX821679) in CI is not considered to be of Austrian origin since it was isolated from a frequent traveler patient (11). The species responsible for old world cutaneous leishmaniasis in these countries are mostly *L. major* and *L. tropica* (12,13). *Leishmania major* causative agent for cutaneous leishmaniasis occurring in the north and east of Iran. Additionally, *Rhombomys opimus* is the reservoir and *Phlebotomus papatasi* is the vector (14). CI strains were isolated from the skin lesion in humans, *P. papatasi* as a vector and *R. opimus* as a reservoir. This high genetic similarity of the strains supports the human-sand fly-rodent cycle in previous studies (15-16). Rafizadeh et al. (2016) reported the human population is associated with *L. major* epidemic, which occurred with more than 400 new CL cases (16). One of the strains sequenced in this study (KJ425408) falls on Clade I in our study. It was also found that this strain was 100% similar to some *L. major* strains (KJ577703, KJ577707, KJ577708) located in Clade I in the Esfarayen region in northern Iran. Several studies from Iran have shown that *L. major* is the dominant species on the border with Afghanistan (17,18). KJ420587 *L. major* strain, originated Herat, occurring in CI is phylogenetically related to strains in Iran.

Clade II (CII) strains belong to *L. infantum* and *L. donovani* in two sub-branches, as seen in Table 2. Several studies reveal that *L. infantum* is a VL agent in the Middle East (19). *Leishmania infantum* origins identified in Uzbekistan, Tajikistan and Armenia were reported to be related to the human and animal migration (20,21). In our study in the CII, Iran *P. tobbi* strain (HQ535858) and Uzbekistan and Armenia human strains are genetically indistinguishable is evidence of the existence of this pathway in terms of transmission. Interestingly, Lin/Argentina/2012 strain detected in the dog in Argentina was also in CII (22). On the other hand, *L.donovani* in Sudan and Ethiopia in CII are form a sub-branch in the phylogenetic tree. The strains of *L. donovani* from Sudan was isolated from clinical samples in 1999 and Ethiopian strains obtained VL and CL cases were located in the same clade (23,24). In this region, people always crossing borders because of doing business or military activities may explain this genotypic relationship. Essentially, according to the current taxonomy, *L. donovani* and *L.infantum* are subspecies in *L. donovani complex* (25).

Clade III (CIII) strains are isolated from different species of desert lizards, human and dog and originated from Afghanistan, China, Iran and Eritrea, in which only strain from Iran is located in CIII (26). Clade IV (CIV) strains are isolated from desert lizards and *Sergentomyia minuta* and originated from China and Spain. It was shown a high similarity that Chine desert lizards strains (KU194923-KU194975) and Spain vector strains (LC216366, LC216368, LC216360) (27,28). It has also been reported that there is a high similarity between desert lizards and human strains in China (29). The reason why Clade III and IV are considered together is that the manifestation of linearity between geographic relations and phylogenetic proximity here is different than expected. Because, while two different clades (CIII and CIV) are detected in the same geography, it is quite remarkable that strains in the remote geography are related to each other."

Herat originated some *L. tropica* (KJ420584, KJ420585) strains belonging to in our study in Clade III (CIII) (30). In our meta-analysis study showed that the KJ420585 *L. tropica* strain isolated from Herat (in CIII) is 100% similar to some Chinese *L. tropica* strains (KU194925, KU194930, KU194937, KU194940). In this study, *L. tropica* Herat strain (KJ420585) was isolated from a human in 2014, however the Chinese strains in

CIII were isolated from some desert lizards (*Eremias vermiculata*) that lived in the north and east of China in 2015 (29). Zhang et al. (2015), reported that desert lizards have a potential reservoir role for human leishmaniasis (28,29). Moreover, some Chinese *L. tropica* strains (KU194928-KU194945, desert lizards, 2015) and Iran strain (HM004586, dog, 2010) were found to be 100% similar in our study. Dogs are known to be the main reservoirs for zoonotic VL (31). In this way, it is possible to see both the historical adventure of *Leishmania* lineages and the transition between species. Herein, strains are geographically related, which can be evaluated to see both the adventure of the origins over the years and the transition between host species. Intrestingly, FN252411 *L. aethiopica* strain in CIII, which is isolated from Eritrea in Africa in 2009, is a human strain. Acording to Zanger et al. (2011), the FN252411 *L. aethiopica* strain was isolated from Eritrea with a travel history (32).

In this study, Clade V's strains are Brazilian and Peru shown as table 2. (FN398328 *L. (V.) shawi* was isolated in 1984 from monkey (*Cebus apella*) while others human cases. They all show 100% similarity to each other, in which monkey species can play a role in the transition path of *Leishmania* species in South America (33). Also, *L. (V.) braziliensis* strains in CVI are 100% similar (34). However, these strains form sub-branches with two other *L. (V.) peruviana* strains isolated from Peru. Cupolillo et al. (1998), supports the genetic closeness between these species also (35). It is interesting that the strains isolated from different hosts, *Leishmania* species and years are located in the same clade. It is understood that genetic profiles are preserved over the years and the ITS1 region is highly similar.

Clade VI has an African Ghanaian strain (EF524071). This strain was named only *Leishmania* sp. taxonomically (36). The *Leishmania* spp. in CVI did not match the DNA sequences of *Leishmania* species in the NCBI portal. EF524071 is isolated from humans and may be a new species, and vector-host relations and epidemiological relations network need to be explained.

In conclusion, the strains examined using the in-silico method were isolated from different geographies of the world between 1984-2018. The phylogenetic relationships between these strains show not only the vertical spread of origins over the years, but also the horizontal spread as geographically. Thus, the relationships of *Leishmania* strains in the host-vector-reservoir chain are explained. Monitoring vertical and horizontal spread of *Leishmania* origins with such meta-analytical studies is particularly important in terms of evaluating the effectiveness of protection and control measures. The demonstration that the horizontal spread of *Leishmania* clones continues between geographies by in-slico and phylogenetic analysis indicates the insufficiency of protection measures. Also, the continuity of the vertical spreading over the years indicates the insufficiency of control measures. Therefore, it is obvious that there is a need for a large number of meta-analysis studies such as this study on factors and their spread.

PRESENTATION AT THE CONGRESS

This article was presented as an oral presentation at the 21st Parasitology Congress (28 September – 3 October 2019, Çeşme, İzmir).

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. World Health Organization. Control of the leishmaniasis: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva. March, 22-26. 2010. http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf (Accessed: December, 2018).
2. Dujardın JC. Risk factors in the spread of leishmaniases: towards integrated monitoring? Trends Parasitol, 2006; 22 (1): 4-6.
3. Teixeira D, Benchimol M, Rodrigues J, Crepaldi P, Pimenta P, De Souza W. The cell biology of Leishmania: how to teach using animations. PLoS Pathog, 2013;9(10): e1003594.
4. Tsukamaya P., Lucas C., Bacon J. Typing of four genetic loci discriminates among closely related species of new world Leishmania. Int. J. Parasit, 2008; 39 (3): 355-62.
5. Zhang C, Lu X, Du X, Jian J, Shu L, Ma Y. Phylogenetic and evolutionary analysis of Chinese Leishmania isolates based on multilocus sequence typing. PLoS One, 2013; 8 (4): e63124.
6. Akopyants NS, Kimblin N, Secundino N, Patrick R, Peters N, Lawyer P, et al. Demonstration of genetic exchange during cyclical development of Leishmania in the sand fly vector. Science, 2009; 324 (5924): 265-8.
7. Akhoundi M, Downing T, Votýpka J, Kuhls K, Lukeš J, Cannet A, et al. Leishmania infections: Molecular targets and diagnosis. Mol Aspects Med, 2017; 57: 1-29.
8. NCBI (Natıonal Center for Biotechnology Information, USA).: www.ncbi.nlm.nih.gov (Accesses: May, 2019).

9. Geneıous Version (11.0.5). https://www.geneious.com/ / (Accessed: December, 2018).

10. Create Maps. 2018. Scribble Maps. https://www.scribblemaps.com/create/ (Accessed: December, 2018).

11. Harrison N, Walochnik J, Ramsebner R, Veletzky L, Lagler H, Ramharter M. Case Report: Progressive Perforation of the Nasal Septum due to Leishmania major: A Case of Mucosal Leishmaniasis in a Traveler. Am J Trop Med Hyg, 2017; 96 (3): 653-5.

12. Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. Expert Rev Anti Infect Ther, 2010; 8 (4): 419-33.

13. Hotez PJ, Savioli L, Fenwıck A. Neglected tropical diseases of the Middle East and North Africa: review of their prevalence, distribution, and opportunities for control. PLos Negl Trop Dis, 2012; 6 (2): e1475.

14. Dobson DE, Kamhawi S, Lawyer P, Turco SJ, Beverley SM, Sacks DL. Leishmania major survival in selective Phlebotomus papatasi sand fly vector requires a specific SCG-encoded lipophosphoglycan galactosylation pattern. PLOS pathogens, 2010; 6 (11): e1001185.

15. Killick-Kendrick R. Phlebotomus vectors of Visceral leishmaniasis, a review. Med Vet Entomol, 4: 1-24.

16. Rafizadeh S, Saraei M, Abaei MR, Oshaghi MA, Mohebali M, Peymani A, et al. Molecular Detection of Leishmania major and L. turanica in Phlebotomus papatasi and First Natural Infection of P. salehi to L. major in North-East of Iran. J Arthropod-Borne Dis, 2016; 10 (2): 141-7.

17. Vaeznia H, Dalimi A, Sadraei J, Pirstani M. Determination of Leishmania spp. causing cutaneous leishmaniasis in Mashhad by PCR-RFLP method. Archives of Razi, 2009; 64 (1): 39-44.

18. Akhoundi M, Hajjaran H, Baghaei A, Mohebali M. Geographical distribution of Leishmania species of human cutaneous leishmaniasis in Fars province, southern Iran. Iran J Parasitol, 2013; 8 (1): 85–91.

19. Rassi Y, Sanei Dehkordi A, Oshaghi MA, Abai MR, Mohtarami F, Enayati A, et al. First report on natural infection of the Phlebtomus tobbi by Leishmania infantum in Northwestern Iran. Exp Parasitol, 2012; 131 (3): 344-9.

20. Alam MZ, Kovalenko DA, Kuhls K, Nasyrova RM, Ponomareva VI, Fatullaeva AA, et al. Identification of the agent causing visceral leishmaniasis in Uzbeki and Tajiki foci by analysing parasite DNA extracted from patients'Giemsa-stained tissue preparation. Parasitology, 2009; 136 (9): 981-6.

21. Sukiasyan A, Keshishyan A, Manukyan D, Melik-Andreasyan G, Atshemyan L, Apresyan H, et al. Re-Emerging foci of visceral leishmaniasis in Armenia-first molecular diagnosis of clinical samples. Parasitology, 2019; 146 (7): 1-8.

22. Acardi SA, Liotta DJ, Santini MS, Romagosa CM, Salomón OD. Detection of Leishmania infantum in naturally infected Lutzomyia longipalpis (Diptera: Psychodidae: Phlebotominae) and Canis familiaris in Misiones, Argentina: the first report of a PCR-RFLP and sequencing-based confirmation assay. Mem Inst Oswaldo Cruz, 2010; 105 (6): 796-9.

23. Gelanew T, Kuhls K, Hurissa Z, Weldegebreal T, Hailu W, Kassahun A, et al. Inference of population structure of Leishmania donovani strains isolated from different Ethiopian visceral leishmaniasis endemic areas. Trop Dis, 2010; 4(11): e889.

24. El Tai NO, Osman OF, El Fari M, Presber W, Schonıan G. Genetic heterogeneity of ribosomal internal transcribed spacer in clinical samples of Leishmania donovani spotted on filter paper as revealed by single-strand conformation polymorphisms and sequencing. Trans R Soc Trop Med Hyg, 2000; 94(5): 575-9.

25. Akhoundi M, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, et al. A Historical Overview of the Classification, Evolution, and Dispersion of Leishmania Parasites and Sandflies. PLoS Negl Trop Dis, 2016;10 (3): e0004349.

26. Mahmoudzadeh-Niknam H, Abrishami F, Doroudian M, Moradi M, Alimohammadian M, Parvizi P, et al. The Problem of Mixing up of Leishmania Isolates in the Laboratory: Suggestion of ITS1 Gene Sequencing for Verification of Species. Iran J Parasitol 2011; 6(1): 41-8.

27. González E, Molina R, Tello A, Iriso A, Vázquez A, Jiménez M. Blood Meal Preferences and Leishmania spp. Detection in Sergentomyia minuta Collected in the Human Leishmaniasis Focus of Madrid, Spain (2012-2016). Conference: 6th World Congress on Leishmaniasis. Poster: May 2017. Toledo/Spain.

28. NCBI: www.ncbi.nlm.nih.gov (last accessed on 10 December 2018).

29. Zhang JR, Guo XG, Liu JL, Zhou TH, Gong X, Chen DL, et al. Molecular detection, identification and phylogenetic inference of Leishmania spp. in some desert lizards from Northwest China by using internal transcribed spacer 1 (ITS1) sequences. Acta Trop, 2016; 162: 83-94.

30. Mosawi S, Dalimi A. Molecular detection of Leishmania spp. isolated from cutaneous lesions of patients referred to Herat regional hospital, Afghanistan. East Mediterr Health J. 2015;21(878-84):12.878.

31. Quinnell RJ, Courtenay O. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. Parasitology, 2009; 136: 1915–34.

32. Zanger P, Kötter I, Raible A, Gelanew T, Schönian G, Kremsner PG. Case Report: Successful treatment of cutaneous Leishmaniasis caused by Leishmania aethiopica with Liposomal Amphothericin B in an İmmunocompromised traveler Returning from Eritrea. Am J Trop Med Hyg, 2011; 84 (5): 692-4.

33. Grimaldi G Jr, Tesh RB. Leishmaniasis of the New World: current concepts and implications for future research. Clin Microbiol Rev, 1993; 6: 230-50.

34. Oddone R, Schweynoch C, Schönian G, de Sousa Cdos S, Cupolillo E, Espinosa D, et al. Development of a multilocus microsatellite typing approach for discriminating strains of Leishmania (Viannia) species. J Clin Microbiol, 2009 Sep;47(9):2818-25.

35. Cupolillo E, Momen H, Grimaldi G Jr. Genetic diversity in natural populations of New World Leishmania. Mem Inst Oswaldo Cruz, 1998; 93: 663-8.

36. Villinski JT, Klena JD, Abbassy M, Hoel DF, Puplampu N, Mechta S, et al. Evidence for a new species of Leishmania associated with a focal disease outbreak in Ghana. Diagn Microbiol Infect Dis, 2008; 60: 323-7.