

## Review Article

# Phlebotomine Sand Flies (Diptera: Psychodidae) in Iran and their Role on *Leishmania* Transmission

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## Abstract

Sand fly research has a long history in Iran beginning with the work of Adler, Theodor and Lourie in 1930 and followed by Mesghali's foundational taxonomic work on sand flies in 1943. Since then, research has been continued unabated throughout the country and official publications report the existence of at least 44 species of sand flies (26 of the genus *Phlebotomus* and 18 of genus *Sergentomyia*) in Iran. So far, seven *Phlebotomus* species and one *Sergentomyia* species have been collected and described by Iranian researchers for the first time. Natural promastigote infections have been repeatedly found in 13 species of sand flies and modern molecular techniques are used routinely to characterize *Leishmania* parasite isolates from endemic areas of cutaneous and visceral leishmaniasis. Because of anthropogenic environmental modifications or human population movements, data on phlebotomine sand flies should be regularly updated and verified at least every five years by fieldwork and taxonomy in foci of leishmaniasis, to incriminate vector species of relevance to the ecology of transmission and to support development and implementation of control programs.

**Keywords:** Sand flies, taxonomy, vectors, *Leishmania major*, *Leishmania tropica*, *Leishmania infantum*, Leishmaniasis, Iran

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## Introduction

Cutaneous and visceral leishmaniasis are ancient endemic diseases in Iran (Maimandi-Nezhad 1965) and continue to be a growing health threat to community development and the environment in the country. Cutaneous leishmaniasis is endemic in two forms, Anthroponotic Cutaneous Leishmaniasis (ACL) and Zoonotic Cutaneous Leishmaniasis (ZCL). About 20,000 cases of leishmaniasis (including ACL, ZCL and Zoonotic Visceral Leishmaniasis) are reported annually but the real figures are 4–5 folds. Anthroponotic cutaneous leishmaniasis is still a neglected tropical disease in many parts of the country. It was greatly reduced in many foci by malaria control measures but many foci remained active in some large and medium sized cities such as

Tehran, Mashhad, Neishabur and Sabzevar in the north-east, Shiraz in the south, Kerman and Bam in the southeast, Yazd, Kashan and parts of the city of Esfahan in the central region (Nadim and Tahvildare-Bidruni 1977, Yaghoobi-Ershadi et al. 2002). The parasite is *Leishmania tropica* and the vector is supposed to be *Phlebotomus (Para-phlebotomous) sergenti* Parrot 1917. The main reservoir host is human but dogs have a role as animal reservoir host and active lesions in dogs have been reported in Tehran, Mashhad, Shiraz and Neishabur (Yaghoobi-Ershadi et al. 2002, Yaghoobi-Ershadi 2008).

Zoonotic cutaneous leishmaniasis is endemic in many rural areas of 17 out of 31

provinces and still is a great health problem in Iran. About 80% of cases reported in the country are of ZCL form (Yaghoobi-Ershadi and Javadian 1995, Yaghoobi-Ershadi et al. 2001b, 2003, Akhavan et al. 2007). *Rhombomys opimus*, the great gerbil, is the main animal reservoir in foci in the north-east and central part of the country, *Meriones libycus*, the Libyan jird, is considered the principal reservoir host in some parts of central and south of the country. *Tatera indica*, the Indian gerbil, is the main reservoir host in the southeast and *Meriones hurrianae*, the desert gerbil, is the reservoir in southeastern part of Iranian Baluchistan, neighboring Pakistan (Yaghoobi-Ershadi and Javadian 1996a, 1996b, Akhavan et al. 2010). *Phlebotomus papatasi* Scopoli 1786, the most prevalent species among *Phlebotomus* genus, is the only known vector (Nadim et al. 1968c, Yaghoobi-Ershadi and Javadian 1997).

Cases of Zoonotic Visceral Leishmaniasis (ZVL) have been reported from all parts of the country (Nadim et al. 1978). About 9,000 parasitological confirmed human cases have been diagnosed and registered from all over the country during the period 1954–2011 (School of Public Health, unpublished data) and most of the cases are under five years old. The epidemiological studies show that the Mediterranean type of Kala-azar occurs in different parts of Iran. Zoonotic visceral leishmaniasis is caused by *Leishmania infantum* LON 49 (Alborzi et al. 2007) and 4 sand fly species belonging to *Larrousius* subgenus and one species of *Paraphlebotomus* subgenus are the probable vectors of ZVL (Yaghoobi-Ershadi 2011). Dogs, foxes, jackals and wolves have been found infected in various parts of the country but dogs are the main reservoir hosts (Navid-Hamidi et al. 1982, Nadim 2008). There are seven endemic foci in the country including Fars Province in the south, Ardabil Province in the northwest, Azarbaijan-e-Sharqi Province in the northwest, Khorramabad area (Lorestan Province) in the west, Khuzestan Province in the southwest and Khorasan-e-

Shomali Province in the northeast. At the moment most of ZVL cases are reported from Fars and Ardabil Provinces which both are tribe areas of Iran (Nadim 2008).

### Historical overview on sand flies from Iran

Phlebotomine sand flies of Iran have been studied since 1930 by a limited number of Iranian and foreign entomologists such as Adler, Theodor and Lourie, who surveyed a small area of the north western part of the country including Kermanshah, Hamadan, Tehran and Rasht. Seven species were found, two of which proved new to science (Adler et al. 1930). Mesghali (1943) was the first Iranian to conduct basic studies on sand flies in Iran. Due to the importance of sand flies and leishmaniasis and the special interest of N. Ansari, Professor and Chairman, Department of Parasitology, University of Tehran, School of Medicine, Mesghali was appointed to carry out a study on the status and distribution of sand flies in Iran. Scientific research had begun at the same time in the country and was greatly facilitated with the establishment of the Institute of Parasitology and Malariology in 1952. Survey teams sent out by this Institute during the preparatory phase of the malaria eradication program or for studying of parasitic diseases were also utilized for the compilation of data on sand flies (Mesghali 1961). Pervomaiski (1948) focused on sand flies in a small area in northern Iran, near the Caspian Sea in the Elburz Mountains. Lewis (1957) collected sand flies in the northeastern part of Iran (Khorassan, Sabzevar area), described a new species, *Phlebotomus ansarii* Lewis 1957 and reported on four species of *Sergentomyia*, three of which represented new country records. Subsequent work by Lewis (1961), Mesghali (1964, 1965, 1968), Theodor (1964), Nadim (1964, 1968, 1970), Javadian (1975, 1997a) and Seyedi-Rashti (1968, 1970) showed that the number of species of both

genera occurring in the country was much more than reported by earlier workers.

In the first general review of the sand fly fauna of Iran, Mesghali (1961) reported 10 *Phlebotomus* and *Sergentomyia* species. In 1976 a key was prepared by Nadim and Javadian in which they reported 19 *Phlebotomus* species and 17 *Sergentomyia* species. In 1990, Seyedi-Rashti produced a pictorial key to 27 *Phlebotomus* and 16 *Sergentomyia* species (School of Public Health, Tehran University of Medical Sciences, unpublished data).

Due to the importance of this subject and the special interest of Iranian researchers, sand fly fauna studies have been updated in recent years with the aid of modern molecular techniques.

### Current times (2007–2011)

In 2007, an intraspecific study on the morphological and molecular characteristics based on mtDNA sequences of *Ph. sergenti* s.l., was performed on 28 Iranian populations from 11 provinces. Three morphotypes were identified as A, B and C. Morphotype A was considered as *Ph. sergenti sergenti*, morphotype B was considered *Ph. sergenti similis*, and morphotype C need further studies (Moin-Vaziri et al. 2007a).

Also in 2007, these same workers carried out a comparative morphological, morphometrical and molecular study on 11 different populations of *Ph. caucasicus* from seven provinces. The results showed that the species includes two morphotypes: C and G. Morphotype C, described as *Ph. caucasicus*, reported in all foci of ZCL. But morphotype G, *Ph. grimmi*, was collected only in three provinces in the northwestern part of the country that are free ZCL (Moin-Vaziri et al. 2007b).

In 2009, the population structure of *Ph. papatasi* was studied based on Multi-locus microsatellite typing of sand flies collected from seven provinces. The results showed

that there are two population variants of *Ph. papatasi*: A<sub>2</sub> from western Iran and A<sub>3</sub> from eastern Iran ((Hamarsheh et al. 2009).

More recently in 2010, a molecular study on sand flies from three Iranian provinces showed *Ph. caucasicus* and *Ph. mongholensis* to be indistinguishable by the mitochondrial cytochrome b gene (Parvizi et al. 2010).

During 2009–2010 a preliminary morphological and morphometric study was performed on two populations of *Ph. major* s.l. from endemic and nonendemic foci of visceral leishmaniasis. The results showed that there were two morphotypes (Badakhshan et al. 2011a). Based on ITS<sub>2</sub> and EF-1 sequences, these two taxa were separated into two well-defined lineages. Moreover, the close similarity of these morphotypes to *Phlebotomus neglectus* specimens deposited by Essegir et al. (2000) in GenBank, was highly informative, suggesting that there are two populations of *Ph. neglectus* in the country (Badakhshan et al. 2011b).

So far eight new species of sand flies including seven *Phlebotomus* species and one *Sergentomyia* species have been collected and described by Iranian researchers for the first time as follows: (i) *Ph. (Syn.) ansarii* Lewis, 1957, (ii) *Ph. (Adl.) brevis* Theodor and Mesghali, 1964, (iii) *Ph. (Lar.) ilami* Javadian, 1997, (iv) *Ph. (Par.) kazeruni* Theodor and Mesghali, 1964, (v) *Ph. (Eup.) mesghalii* Rashti and Nadim, 1970, (vi) *Ph. (Eup.) nadimi* Javadian, 1997, (vii) *Ph. (Phl.) salehi* Mesghali and Rashti, 1968, (viii) *Se. (Par.) iranicus* Lewis and Mesghali, 1961.

### Current composition of sand fly fauna of Iran

National and international publications on sand flies show that in Iran the number of sand fly species account for a total of 44 (Table 1). Sand fly fauna include 26 *Phlebotomus* species of 6 subgenera and 18 *Sergentomyia* species of 6 subgenera. *Phlebotomus sergenti sergenti* and

*Ph. sergenti similis* reported in Table 1 are not counted, being considered subspecies.

The presence of further seven species including: *Ph. (Adl.) kabulensis* Artemiev, 1978, *Ph. (Adl.) salangensis* Artemiev, 1978, *Ph. (Lar.) langeroni* Nilzulesco, 1930, *Ph. (Transphlebotomus.) mascittii* Grassi, 1908, *Ph. (Lar.) smirnovi* Perfiliew, 1941, *Ph. (Eup.) caudatus* Artemiev, 1978, *Ph. (Adl.) turanicus* Artemiev, 1974 -doubtful in the country, because Iranian senior sand fly specialists have not confirmed them yet and must be verified by further studies.

Most entomologists believe that the sand flies of Iran are predominantly Palearctic, with Mediterranean elements entering from the northwest and central Asiatic elements from the northeast. The area along the Persian Gulf contains both Indian and African elements entering through southern Arabia (Theodor and Mesghali 1964). The emergence of new sand fly populations in Iran may have resulted from the introduction recently or historically with people who settled in the agricultural areas stretching from the Nile River to the southern coast of the Mediterranean Sea, around the Syrian Desert and north of Saudi Arabia and Yemen to the Persian Gulf. Known as the Fertile Crescent, this area has an impressive record of past human activity and transportation, including migrations of some of the earliest known peoples (Hamarshah et al. 2009).

### **Studies on *Leishmania* vector incrimination**

Studies on the vectors of various *Leishmanias* in Iran began in 1964 and continue, especially in newly discovered foci. Natural promastigote infections have been found repeatedly in 13 species of sand flies (10 *Phlebotomus* and 3 *Sergentomyia* species).

#### ***Phlebotomus sergenti* s.l.**

Natural promastigote infections have been

found in this species in two ACL foci due to, Mashhad in the northeast with an infection rate of 1.5% (Mesghali et al. 1967) and Esfahan City in central Iran with an infection rate of 0.1 % (Nadim and Seyedi-Rashti 1991). *Phlebotomus sergenti* was reported as *L. tropica* host by PCR in Shiraz City, south of Iran (Oshaghi et al. 2010). Two (66.6%) out of 3 *Ph. sergenti* females have been found infected with *Leishmania turanica* based on PCR detection of parasite ITS2-rDNA in the city of Bushehr, southwestern Iran in 2011 (Yaghoobi-Ershadi, unpublished data). *Phlebotomus sergenti* has a wide distribution in the country that includes and extends beyond the distribution of *L. tropica*.

#### ***Phlebotomus papatasi***

The main vector of *Leishmania major* to humans and gerbils is *Ph. papatasi*. In almost all ZCL foci natural promastigote infections have been found in this species, ranging from 0.2 to 10.9% in flies captured from rodent burrows during 1967–1990 with an average infection rate of 5.6%. (Yaghoobi-Ershadi and Akhavan 1999, Yaghoobi-Ershadi et al. 2001c). In 1991, *Leishmania* promastigotes isolated from this species in central Iran, they were typed at the faculty of Medicine in Montpellier by the examination of 15 isoenzymes and identified as *L. major* zymodeme MON-26 (Yaghoobi-Ershadi et al. 1995b).

Natural infections with *Leishmania* promastigotes were monitored in sand flies from rodent burrows in Borkhar in central Iran from late June to mid October 1991. *Phlebotomus papatasi* infections appeared at the end of July peaked in September with a rate of 17.3% and fell to zero early in October (Yaghoobi-Ershadi and Javadian 1996b). Specimens of this species collected from rodent burrows in villages of Badrood, in central Iran in 1998 were also found to be naturally infected with *L. major* zymodeme MON-26. Promastigote infection rates varied between 6.7–22% during the sand

fly season, being greatest in September, coincident with second activity peak of *Ph. papatasi*, 2–3 months before the highest incidence of human ZCL cases in November–December. The promastigote infection rate was 1.1% in 94 *Ph. papatasi* in indoors (Yaghoobi-Ershadi et al. 2001a).

During the last decade on many occasions *L. major* isolates from *Ph. papatasi* have been detected and identified by Nested-PCR, Semi Nested PCR and Rapid-PCR or isoenzyme characterization in different foci of ZCL in the country (Yaghoobi-Ershadi and Akhavan, 1999, Yaghoobi-Ershadi et al. 2001c, Rassi et al. 2008, Oshaghi et al. 2008). Parvizi and Ready (2008) conducted surveys in two foci of ZCL in Esfahan and Golestan Provinces located in the central and northeastern parts of the country respectively, detected and identified *L. major*, *L. turanica* and *Leishmania gerbilli* in *Ph. papatasi* collected from gerbil burrows and animal shelters using Nested-PCR and sequencing of nuclear ITS-rDNA fragments. This species plays the key vectorial role in the transmission cycle of ZCL due to *L. major* in many rural areas of 17 out of 31 provinces of the country (Fig. 1).

### ***Phlebotomus caucasicus*, *Phlebotomus mongolensis* and *Phlebotomus andrejevi***

Besides *Ph. papatasi*, the three species, *Ph. caucasicus*, *Ph. mongoloensis* and *Ph. andrejevi*, are believed to maintain the enzootic cycle of *L. major* among gerbils and jirds. The females of these sand flies are not distinguishable morphologically. Natural promastigote infection rates in *Ph. caucasicus* from 3.3–20% have been reported in specimens collected from rodent burrows, with frequent observation of parasites in the anterior midgut and head (Yaghoobi-Ershadi and Javadian 1996b, 1997). In 1994, promastigotes were isolated from heavily infected *Ph. caucasicus* collected from gerbil and jird burrows in the main focus of Esfahan and typed by

isoenzymes as *L. major* zymodeme MON-26 at the WHO *Leishmania* reference Center, Faculty of Medicine, University of Montpellier, France (Yaghoobi-Ershadi et al. 1994). The same *Leishmania* species was isolated again from *Ph. caucasicus* collected in gerbil burrows in Jarghooyeh rural district, southeastern Esfahan in 1997 (Yaghoobi-Ershadi et al. 2001c).

In 2008, *L. major* and *Leishmania near gerbilli* were detected and identified in *Ph. caucasicus* by Nested PCRs and DNA sequence analysis from specimens collected in rodent burrows and animal shelters in Esfahan, central Iran and Golestan Province in northeastern Iran respectively (Parvizi and Ready 2008). These findings combined with a human blood index of 20% provide strong evidence that *Ph. caucasicus* is indeed a natural vector of *L. major* in central Iran and a secondary vector to humans in the country (Yaghoobi-Ershadi et al. 1995a).

### ***Phlebotomus ansarii***

This species has been found so far only in Iran. Several studies have reported infection rates ranging from 3.7–11.5% in specimens collected from rodent burrows in the Esfahan focus of ZCL central Iran (Nadim et al. 1968c, Yaghoobi-Ershadi and Javadian 1996). In 2008 *L. near gerbilli* was identified by Nested PCR of ITS-rDNA in specimens of this species collected from gerbil burrows in Esfahan (Parvizi and Ready 2008).

### ***Phlebotomus salehi***

This species is considered to be a vector in the enzootic cycle of *L. major* among gerbils and has been collected in rodent burrows on the plain of Chabahar in Baluchistan and also Hormozgan Provinces in southern Iran (Mesghali 1965, Mesghali and seyedi-Rashti 1968). The average natural promastigote infection rate in specimens collected from rodent burrows in southeastern Iran was 1.07% in 1997 (Kasiri and Javadian 2000).

**Table 1.** List of sand fly species recorded in Iran

Genus	Sub-genus	Species	
<i>Phlebotomus</i>	<i>Adlerius</i> Nitzulescu, 1931	1. <i>Ph. (Adl.) balcanicus</i> Theodor, 1958	
		2. <i>Ph. (Adl.) brevis</i> Theodor and Mesghali, 1964	
		3. <i>Ph. (Adl.) halepensis</i> Theodor, 1958	
		4. <i>Ph. (Adl.) longiductus</i> Parrot, 1928	
	<i>Euphlebotomus</i> Theodor, 1948	5. <i>Ph. (Eup.) mesghalii</i> Seyedi-Rashti and Nadim, 1970	
		6. <i>Ph. (Eup.) nadimi</i> Javadian, 1997	
	<i>Larroussius</i> Nitzulescu, 1931	7. <i>Ph. (Lar.) ilami</i> Javadian, 1997	
		8. <i>Ph. (Lar.) kandelakii</i> Shchurenkova, 1929	
		9. <i>Ph. (Lar.) keshishiani</i> Shchurenkova, 1936	
		10. <i>Ph. (Lar.) neglectus</i> Tonmir, 1921	
		11. <i>Ph. (Lar.) perfilevi</i> Perfilev, 1937	
		12. <i>Ph. (Lar.) tobbi</i> Adler and Theodor, 1930	
		13. <i>Ph. (Lar.) wenyoni</i> Adler and Theodor, 1930	
<i>Paraphlebotomus</i> Theodor, 1948	14. <i>Ph. (Par.) alexandri</i> Sinton, 1928		
	15. <i>Ph. (Par.) andrejevi</i> Shakirzyanova, 1953		
	16. <i>Ph. (Par.) caucasicus</i> Marzinowsky, 1917		
	17. <i>Ph. (Par.) grimmi</i> Porchinsky, 1876		
	18. <i>Ph. (Par.) jacusieli</i> Theodor, 1947		
	19. <i>Ph. (Par.) kazeruni</i> Theodor and Mesghali, 1964		
	20. <i>Ph. (Par.) mongolensis</i> Sinton, 1928		
	21. <i>Ph. (Par.) sergenti</i> s.l. Parrot, 1917		
	<i>Ph. sergenti sergenti</i> Parrot 1917		
	<i>Ph. segenti similis</i> Perfilew, 1963		
	<i>Phlebotomus</i> Rondani, 1840	22. <i>Ph. (Phl.) bergeroti</i> Parrot, 1934	
23. <i>Ph. (Phl.) papatasi</i> (Scopoli), 1786			
24. <i>Ph. (Phl.) salehi</i> Mesghali and Rashti, 1968			
<i>Synphlebotomus</i> Theodor, 1948	25. <i>Ph. (Syn.) ansarii</i> Lewis, 1957		
	26. <i>Ph. (Syn.) eleanorae</i> Sinton, 1931		
<i>Grassomyia</i> Theodor, 1958	27. <i>Se. (Gra.) dreyfussi</i> Parrot, 1933		
	28. <i>Se. (Gra.) squamipleuris</i> (Newstead), 1912		
<i>Parrotomyia</i> Theodor, 1958	29. <i>Se. (Par.) africana</i> Newstead, 1912		
	30. <i>Se. (Par.) baghdadis</i> (Adler and Theodor), 1929		
	31. <i>Se. (Par.) grekovi</i> (Khodukin), 1929		
	32. <i>Se. (Par.) palestinensis</i> (Adler and Theodor), 1927		
	33. <i>Se. (Par.) sogdiana</i> (Parrot), 1929		
	34. <i>Se. (Par.) sumbarica</i> (Perfilev), 1933		
<i>Sergentomyia</i>	<i>Parvidens</i> Theodor and Mesghali, 1964	35. <i>Se. (Par.) iranicus</i> Lewis and Mesghali, 1961	
		<i>Rondonomyia</i> Theodor, 1958 <i>Neophlebotomus</i> Franca and Parrot, 1920	36. <i>Se. (Ron.) pawlowskyi</i> (Perfilev), 1933
	<i>Sergentomyia</i> Franca and Parrot, 1920		37. <i>Se. (Ser.) antenata</i> Newstead, 1912
			38. <i>Se. (Ser.) dentata</i> Sinton, 1933
		39. <i>Se. (Ser.) mervynae</i> Pringle, 1953	
		40. <i>Se. (Ser.) theodori</i> (Parrot), 1942	
		41. <i>Se. (Ser.) sintoni</i> Pringle, 1933	
	<i>Sintonius</i> Nitzulescu, 1931	42. <i>Se. (Sin.) christophersi</i> (Sinton), 1927	
		43. <i>Se. (Sin.) tiberiadis</i> (Adler, Theodor and Lourie), 1930	
		44. <i>Se. (Sin.) clydei</i> (Sinton), 1928	

**Table 2.** Proven or suspected vector species present in Iran, *Leishmania* agent transmitted and endemic provinces

Proven or suspected vector	<i>Leishmania</i> agent transmitted	Province	References
<i>Ph. papatasi</i> *	<i>L. major</i>	Esfahan	(Nadim et al. 1968c), (Yaghoobi-Ershadi et al. 1995, 2001b)
		Khorasan-e-Razavi	(Mesghali et al. 1967), (Yaghoobi-Ershadi et al. 2003)
		Khorasan-e-Shomali	(Javadian et al. 1976)
		Golestan	(Nadim et al. 1968b), (Parvizi and Ready 2008)
		Yazd	(Yaghoobi-Ershadi et al. 2004, 2007)
		Kerman	(Yaghoobi-Ershadi, 2010), (Akhavan et al. 2007)
		Khuzestan	(Javadian et al. 1977b), (Javadian and Ranjbar, 1990)
		Qom	(Akhavan et al. 2003), (Rassi et al. 2011b)
		Tehran	(Seyedi-Rashti and Salehzadeh, 1990)
		Sistan and Baluchestan	(Seyedi-Rashti and Nadim, 1984a), (Kasiri and Javadian, 2001)
		Hormozgan	(Hanafi-Bojd et al. 2007)
		Bushehr	(Yaghoobi-Ershadi, unpublished data)
		Fars	(Rassi et al. 2007), (Azizi et al. 2010)
		Semnan	(Rassi et al. 2011a)
		Ilam	(Javadian and Ranjbar, 1990)
		<i>Ph. papatasi</i>	<i>L. turanica</i>
Golestan	(Parvizi and Ready, 2008)		
Semnan	(Rassi et al. 2011a)		
	<i>L. near gerbilli</i>	Esfahan	(Parvizi and Ready, 2008)
<i>Ph. sergenti</i>	<i>L. tropica</i>	Khorasan-e Razavi	(Mesghali et al. 1967), (Nadim and Seyedi-Rashti, 1991)
		Esfahan	(Javadian and Seyedi-Rashti, 1991)
		Yazd	(Yaghoobi-Ershadi et al. 2002)
		Kerman	(Yaghoobi-Ershadi 1977), (Seyedi-Rashti et al. 1984b), (Nadim and Aflatoonian, 1995)
		Fars	(Nadim and Seyedi-Rashti, 1971), (Oshaghi et al. 2010)
		Tehran	(Nadim and Seyedi-Rashti, 1991)
	<i>L. near gerbilli</i>	Esfahan	(Parvizi and Ready, 2008)
<i>Ph. caucasicus</i> <i>Ph. mongolensis</i> <i>Ph. andrejevi</i>	<i>L. major</i>	Esfahan	(Yaghoobi-Ershadi et al. 1994, 1995a, 2001c), (Parvizi and Ready, 2008)
		Esfahan	(Parvizi and Ready, 2008)

**Table 2.** Countinued...

<i>Ph. ansarii</i>	<i>L. major</i>	Esfahan	(Nadim et al. 1968c), (Yaghoobi-Ershadi and Javadian, 1996)
	<i>L. near gerbilli</i>	Esfahan	(Parvizi and Ready, 2008)
<i>Ph. salehi</i>	<i>L. major</i>	Sistan and Baluchestan	(Kasiri and Javadian, 2001)
<i>Ph. alexandri</i>	<i>L. infantum</i>	Fars	(Azizi et al. 2006)
	<i>L. major</i>	Khuzestan	(Javadian et al. 1977)
<i>Ph. neglectus</i> (= <i>Ph. major</i> s.l.)	<i>L. infantum</i>	Fars	(Sahabi et al. 1992), (Azizi et al. 2008)
<i>Ph. keshishiani</i>	<i>L. infantum</i>	Fars	(Seyedi-Rashti et al. 1995)
<i>Ph. kandelakii</i>	<i>L. infantum</i>	Ardabil	(Nadim et al. 1992), (Rassi et al. 2005)
<i>Ph. perfiliewi</i>	<i>L. infantum</i>	Ardabil	(Nadim et al. 1992), (Rassi et al. 2009)
	<i>L. donovani</i>	Ardabil	(Oshaghi et al. 2009)
	<i>L. infantum</i> <i>L. tropica</i>	Azarbaijan-e-Sharqi	(Parvizi et al. 2008)

\* Sand fly species marked with asterisk are proven vectors according to the generally accepted criteria for incriminating *Leishmania* vectors (Killick-Kendrick and Ward 1981, WHO 2010). Species with no asterisk are suspected to be vectors on the basis of epidemiological evidence or because are proven vectors elsewhere.



**Fig. 1.** Map of Iran providing the province outlines, in brown the provinces that are endemic for zoonotic cutaneous leishmaniasis



### ***Phlebotomus alexandri***

This species is usually found in mountainous areas and has been reported all over the country. In field studies conducted during 1971–1972, a natural promastigote infection rate of 1.7% was reported in specimens collected from rodent burrows in Khuzestan Province, southwestern Iran (Javadian et al. 1977) but isolation and characterization of the parasite from the sand fly vector has not been achieved yet in the study area. In 2006 promastigotes isolated from *Ph. alexandri* collected from the houses of ZVL cases in Nourabad Mamasani district, Fars Province, south of the country, were identified as *L. infantum* by semi-Nested PCR. The natural infection rate was 4.2% in this species, strongly implicating it as probable vector of ZVL (Azizi et al. 2006).

### ***Phlebotomus neglectus* Tonnoir, 1921 (= *Ph. major* s.l.)**

This species is found in 17 of 31 provinces mostly in mountainous areas and has been found in all areas from which human cases of ZVL have been reported. In 1992, the promastigote infection rate in specimens collected outdoors by light traps was reported to be 3% and by aspirator from indoors it was 5% in Ghir-Karzin district, another endemic focus of ZVL in Fars Province, southern Iran but the parasites were not characterized (Sahabi et al. 1992). In 2008, the infection rate in flies collected from the same area was 8.3% and parasite isolates were characterized and identified by Nested-PCR as *L. infantum*, implicating this species as a probable vector of ZVL in the south of the country (Azizi et al. 2008).

### ***Phlebotomus keshishiani***

In 1995, during a sand fly survey conducted in an endemic ZVL focus in Ghir-Karzin, Fars Province, southern Iran, the natural promasti-

gote infection rate in specimens of this species collected outdoors was 1.1%, so the species is also considered a probable vector of ZVL (Seyedi-Rashti et al. 1995).

### ***Phlebotomus kandelakii***

In a 1991–1992 field study in the endemic focus of Meshkin-Shahr in northwestern Iran, the natural *Leishmania* infection rate in this species was reported to be 0.3% (Nadim et al. 1992). Similar studies were conducted in the same area from 2001–2005 in which the *Leishmania* infection was reported to be 1.1%. Parasite isolates from these later studies were characterized and identified as *L. infantum* by nested PCR (Rassi et al. 2005). Based on these findings, *Ph. kandelakii* is considered a probable vector of ZVL in northwestern Iran.

### ***Phlebotomus perfiliewi***

In an entomological investigation carried out between 1991–1992 in Germi County, northwestern Iran, the natural promastigote infection rate was 0.9% in this species (Nadim et al. 1992). In a later study conducted in the same area between 2004–2005 the infection rate was 1.1% and isolates were characterized and identified as *L. infantum* by PCR, incriminating the species as a probable vector of ZVL (Rassi et al. 2009). More recently in 2006, *L. infantum* and *L. donovani* were detected and identified by PCR and DNA sequencing in from specimens collected from the same area. Of seven isolates, one was *L. infantum* and the other six were haplotypes of *L. donovani* (Oshaghi et al. 2009).

It should be mentioned that the parasites from *Ph. kandelakii*, *Ph. perfiliewi transcaucasicus*, *Ph. keshishiani* and *Ph. neglectus* were injected intraperitoneally into hamsters and in all cases produced heavy visceral infections but the investigators failed to keep them in culture (Nadim et al. 1992).

Table 2 shows the list of proven or suspected vector species present in Iran.

### Vectors of Lizard leishmaniasis (*Sauroleishmania*)

Lizard leishmaniasis is also widespread in four species of lizards in various areas of Iran as follows:

*Agama agilis* and *Agama melanura* collected either from gerbil burrows or lizard holes in dry river beds in Khorassan-e-Razavi and Golestan Provinces in the northeast, *Agama caucasica* from Manjil area in Qazvin Province (the mid north of the country) and *Cyrtopdion caspius* from Turkmen-Sahra in Golestan Province, the north of the country (Nadim et al. 1968a, 1968b, Seyedi-Rashti et al. 1971). In all areas where lizard leishmaniasis has been found it has been accompanied by natural infections in *Sergentomyia* species.

#### *Sergentomyia sintoni*

In 1966 in a sand fly survey conducted in several ZCL foci in northeastern Iran, many *Se. sintoni* specimens were found naturally infected with promastigotes (Nadim et al. 1968b). From 1967–1995 frequent promastigote infections in this species were reported frequently in ZCL foci throughout the country (Yaghoobi-Ershadi and Akhavan 1999). Isolates from this sand fly species were identified as *L. (Sauroleishmania) gymnodactyli* by isoenzyme analysis (Seyedi-Rashti et al. 1994). In 2008, isolates from natural promastigote infections in flies from the central part of the country were identified as *L. major* and *L. near gerbilli* by sequencing of the ITS1 or ITS2 microsatellite fragment or sizing of the ITS2 microsatellite fragment (Parvizi and Ready 2008).

#### *Sergentomyia clydei*

In 1967, promastigote infections in this species, thought to be related to lizard leish-

maniasis, were reported in sand fly specimens collected from gerbil colonies in Lotf-Abad (Khorasan-e Razavi Province) in the northeastern part of the country (Mesghali et al. 1967).

#### *Sergentomyia dentata*

In 1994, natural promastigote infections in this species were reported during an entomological survey in the northwestern part of the country (Rassi et al. 1997).

### Concluding remarks

Relevant studies of leishmaniasis are impossible without correct identification of the associated sand flies and a thorough understanding of their distribution and biology. Over the past 80 years many researchers have made significant contributions to the body of knowledge implicating various *Leishmania* vectors in Iran.

The likelihood of finding natural *Leishmania* infections in sand flies is related to the age of the sand fly vector and most of the dissections were carried out during August–September of the years of studies towards to the end of the active season of sand flies in Iran. This is because the risk of infection is nil when the new generation of a sand fly vector begins to emerge, but becomes increasingly high as the population ages and more parous flies are present. This variation is not as marked with vectors that are present throughout the year in other parts of the world as in the Neotropics for example. The other important point is the detection of *Leishmania* DNA in a sand fly which does not prove that it is a vector. For example, the PCR assays did not distinguish between infective stage metacyclic parasites and non-infective stages of *Leishmania* promastigotes. The generally accepted criteria for incriminating *Leishmania* vectors are: anthropophily in transmission habitats and disease foci, devel-

opment in the sand fly of typed infections through its complete extrinsic life cycle, and experimental transmission by bite (Killick-Kendrick and Ward 1981). It should be mentioned that members of WHO have stated in 2010 that existing knowledge and molecular tools for the identification and characterization of *Leishmania* parasites and sand flies should be improved and harmonized (WHO 2010).

Data on phlebotomine sand flies should be regularly updated and verified at least once every five years by fieldwork and taxonomy in leishmaniasis foci, because of increasing environmental modifications or population movements which are important in preparing strategic plan of leishmaniasis control. Further research is needed to amplify present knowledge and extend it to areas not yet studied. Research efforts in the field are continuously discovering new data on sand flies in the world and it is the duty of sand fly specialists to remain abreast of these developments.

Long-term monitoring should be conducted of annual fluctuations in important vector populations in representative transmission sites of leishmaniasis in Iran. Examples of vectors to be included in this research are *Ph. papatasi*, *Ph. sergenti*, *Ph. kandelakii*, *Ph. neglectus* and *Ph. alexandri*.

Careful incrimination of other vectors in different endemic areas and clarification of the ecology concerning transmission and control means that long term efforts are needed in the future. And this kind of knowledge requires full support and more funding for the field work by health authorities.

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