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Ecological habitat of small mammals as reservoirs of *Leishmania* spp. in the endemic foci of cutaneous leishmaniasis, the north Iran

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Abstract
Small mammals are invasive species to agriculture and food resources worldwide. They are also reservoir hosts for medically and veterinary important zoonotic pathogens worldwide. Identifying ecological environments that serve as different small mammals' habitats as reservoirs for vector-borne diseases can help us to restrain their populations and manage the place of risk for spreading zoonotic diseases. This study was aimed to collect and identify the small mammals in northern Iran and to investigate the habitats with more potential risk for reservoirs of zoonotic *Leishmania*. In this survey, 294 specimens of small mammals were collected from the north of Iran between 2019 to 2023. Additionally, the abundance of zoonotic hosts and the rate of *Leishmania* prevalence reports in small mammals during the last ten years were analyzed to detect the relationship between reservoir distribution and parasitic infection. The collecting specimens belong to 12 families, 20 genera, and 26 species, and the most variety of species were collected from forest areas, while the lowest (4 species) were caught from urban and rural areas. According to the relative abundance index, the four most abundant small mammal species were *Rhombomys opimus*, *Rattus norvegicus*, *Mus musculus*, and *Microtus obscurus*. *Rhombomys opimus*, *R. norvegicus*, and *Meriones libycus* exhibit the highest levels of infection. This research, suggests comprehensive studies to detect *Leishmania* infection to determine new reservoirs in small mammals.

Introduction

The north of Iran features a diverse range of ecological habitats like Hyrcanian forests, wetlands, and mountainous areas. Hyrcanian forests comprise a continuous 800-kilometers-belt of deciduous forests located between the Caspian Sea and the Alborz Mountains, with an area of over 1.8 million hectares (1). These forests were long known as a biodiversity hotspot (Hyrcanian hotspot) and past refugia for several groups especially small mammals including orders Rodentia, Lagomorpha, Eulipotyphla, Chiroptera, and some species of Carnivora (2-5). Hyrcanian forests and the Alborz Mountains host a high diversity of small mammals (3, 5, 6). Small mammals such as rodents, bats, insectivores, hares, and some species of carnivores

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have been known as hosts and/or reservoirs of *Leishmania* spp. in the world. Iran is also an endemic country for leishmaniasis (7-10). Leishmaniasis is one of the vector-borne diseases caused by obligate intracellular parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) that has been a severe health problem in tropical and subtropical areas including 98 endemic countries in the world and can infect over one billion people in those countries yearly (11). Leishmaniasis can be into Old World and New World Leishmaniasis. Old World Leishmaniasis can lead to Visceral Leishmaniasis (VL), caused by *Leishmania infantum* and *L. donovani,* and cutaneous leishmaniasis (CL), caused by *Leishmania major, L. tropica,* and *L. aethiopica* (12). This disease is transmitted by female sandflies (Diptera: Psychodidae: *Phlebotomus*) (13). Iran is one of the countries with the highest annual morbidity of Leishmaniasis in the world. On average, 20,000 new reports of CL are reported in Iran by the Ministry of Health per annum, and due to the high prevalence and endemicity of Cutaneous Leishmaniasis, the disease is one of the major community health problems (14, 15).

Additionally, rodents are known to remain asymptomatic carriers of *Leishmania* spp. for very long periods. Consequently, it can be hypothesized that rodent populations, as well as other wild animals, can maintain the permanent circulation of the parasite in an endemic area (16). The favorable ecological conditions and abundance of small mammals in the north of Iran, permit *Leishmania* species to spread into new geographical areas. Therefore, it is necessary to investigate possible species of reservoirs of *Leishmania* and their role in transmission.

Various species of small mammals such as *Rhombomys opimus*, *Nesokia indica*, *Meriones libycus, M. persicus, M. hurrianae, Tatera indica, Gerbillus nanus*, *Rattus rattus*, *R. norvegicus*, *Mus musculus*, and *Hemiechinus auritus* have been identified as potential reservoir hosts of the CL in Iran (8, 17, 18).

Some species of small mammals have been reported only from the north of Iran like *Crocidura capsica*, *Eptesicus nilssonii*, and *Martes martes* (3). This shows the diversity and uniqueness of the ecological habitats of northern Iran. The report of some species such as *R. opimus*, *M. libycus*, *N. indica*, *R. rattus*, *R. norvegicus*, and *M. musculus* as reservoirs of *Leishmania* and the presence of species of sand fly like *Phlebotomus papatasi* and *Phlebotomus sergenti* as *Leishmania* transmission agent, for instance in Golestan Province, in the northwest of Iran, *R. opimus sodalist* subspecies has been reported as a reservoir of Cutaneous Leishmaniasis (8) indicates the potential of other species of small mammals as a reservoir of *Leishmania* sp. in the north of Iran.

Distribution and community assemblage in small mammals have changed by anthropogenic activities in the area; therefore zoonotic diseases such as Leishmaniasis are dramatically increased. Recent studies show there is a significant correlation between the incidence of Cutaneous Leishmaniasis, the number of rodent burrows, the frequency of *Ph. papatasi* and geo-climatic factors (18, 19). For example, sand fly (*Ph. papatasi*) as the main vector of *L. major* has been collected from near rodent burrows such as *R. opimus, M. libycus, M. persicus,* and *Hemiechinus auritus* in Golestan Province, Iran (8, 17).

A variety of ecological habitats in the north of Iran supports a diverse population of mammalian species, making them potential hosts for *Leishmania* species. Therefore, studying their distribution and diversity is of critical importance. This study aimed to investigate the potential role of small mammals as reservoirs of *Leishmania* in northern Iran and elucidate the interrelations between wildlife biodiversity and dynamics of zoonotic disease in the north of Iran.

Materials and methods

Study area

The north of Iran includes different habitats, such as forests, mountains, and plains, which cause species richness higher than other parts of Iran. This study was conducted in the eastern parts of the Hyrcanian region and the Elborz Mountains, spanning an elevation gradient from −20m (asl) to 3000m (asl) from September 2019 to June 2023. The study area was partitioned into three elevation levels according to vegetation coverage: the first level ranged from −20 to 500 m, encompassing farms and areas adjacent to wetlands; the second level spanned from 500 to 1700 m, covering deciduous forests of the Elborz Mountains range, mixed broadleaf forests, pasturage and coniferous scrublands; the third level stretched from 1700 to 3000 m, including the rocky areas of the Elborz Mountains. Sampling was conducted over 200 trap nights that included all three elevation levels.

Sampling

To investigate the present or absence of hosts as *Leishmania* reservoirs in northern Iran, 294 small mammals were captured using live traps and snack baits, pitfalls, and mist-net across five ecological habitats defined in this study including urban and rural areas, fields, and gardens, plain, forest, and mountainous areas (Table 1 and Figure 1). The snack, cucumber, and nuts were used as baits. The traps were placed at each station for a night and checked early in the morning. The captured specimens were collected in the morning and then were considered from sexed, weighed, and measured externally, and finally sacrificed specimens were fixed in ethanol 96%. The specimens were identified using diagnostic characters and identification keys available in the literature (20). ArcGIS ver.10.8 was used to prepare maps of the sampling area.

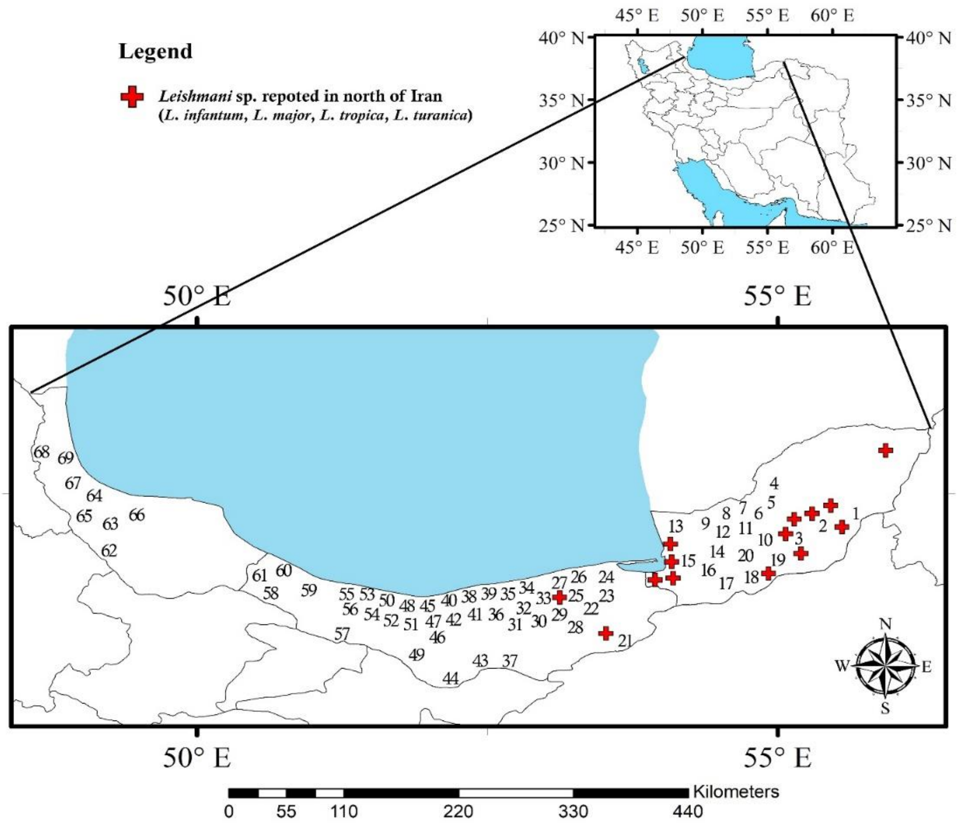

Fig. 1. Sampling map of small mammals and distribution of *Leishmania* in the small mammals and humans in the north of Iran. The specimens collected from 41 regions are marked by a number and reports of *Leishmania* in the small mammals and humans are marked by a plus on the map.

Statistical analyses

The abundance of zoonotic hosts and the rate of Leishmaniasis prevalence were investigated to detect the relationship between host and parasite diversity. Host abundance was estimated as the total number of individuals captured per total capture as follows, in which the number captured by each species (total number of individuals captured) was divided by the total number of samples captured. We used the Web of Science to search for papers that reported Leishmaniasis in the north of Iran from 2013 to 2023 (Appendix). We examined experimental or observational studies between host quality and the presence of parasites in this area. Therefore, we used Analysis of Variance (ANOVA) to examine whether the host distributional range has a statistically significant effect on Leishmaniasis infection prevalence (P-value < 0.05), also the abundance of each species was assessed using the relative abundance index. All statistical analyses were performed utilizing SPSS version 26.0 (SPSS, Chicago, IL, USA).

Results

Diversity and abundance of small mammals

In total, 294 specimens of small mammals belonging to 12 families, 20 genera, and 26 species were recorded and identified from the margins of the Caspian Sea in the north of Iran from September 2018 to June 2023 (Tables 1 and 2). The most prevalent species were *R. norvegicus*, *M. musculus*, and *M. obscurus*. According to the relative abundance index, *R. norvegicus*, representing 48 samples with an abundance of 16.43% among all samples, was found in four ecological habitats: plain areas, urban and rural areas, forest areas, and fields and gardens (Table 2 and Figure 2). *Microtus obscurus* and *M. musculus* were the second most abundant species with an abundance of 14.38% (42 specimens for each species). *Microtus obscurus* and *Calomyscus grandis* are new records for Gilan and Mazandaran Provinces respectively and were found in plain and mountainous areas (Table 2 and Figure 3), whereas *M. musculus* was found in forest and urban areas. The species *A. hyrcanicus*, *C. grandis*, *H. indica* (the order Rodentia), and *P. kuhlii* (order Chiroptera) were rare in numbers, with an abundance of one specimen (0.34%) among all samples. *Apodemus hyrcanicus*, *H. indica*, and *P. kuhlii* were found in forest areas, while *C. grandis* was only observed in mountainous areas, with an elevation of over 2500 meters (Table 2 and Figure 3). *Calomyscus grandis*, *A. hyrcanicus*, and *Glis persicus* were endemic species of the research area. The highest number of species (16 species) were obtained from forest areas, while the lowest (4 species) were obtained from urban and rural areas (Figure 3). Therefore, the forest habitat type had the highest number of species and number of individuals, followed by the two ecological habitats of gardens and plants with 15 species.

Records of small mammals as host of Leishmania spp. in the north Iran

From 2013 to 2023, 1056 samples of small mammals were captured and investigated from the north of Iran, of which 446 samples were infected. *Leishmania* infection was observed in Rodentia and Eulipotyphla specimens. *Rhombomys opimus*, *M. libycus*, *N. indica,* and *M. musculus* exhibit the highest levels of infection in north Iran.

The infection rates were investigated in the identified reservoirs in north Iran by species (CI = confidence interval: 95%; Table 3). These studies suggest that other small mammals, especially other rodents and eulipotyphla species, could also serve as potential *Leishmania* reservoirs in north Iran. The infection rates estimated in this survey were just significant for *M. persicus* (17.26-62.35%; CI = confidence interval: 95%; Table 3) meaning that the number of *M. persicus* sampled and the percentage of infection to the *Leishmania* parasite is not independence. The previous studies suggest that other small mammals, especially other rodent species e.g., could also serve as potential *Leishmania* reservoirs in northern Iran.

According to several studies from 2013 to 2023, four *Leishmania* species: *L. major*, *L. infantum*, *L. tropica*, and *L. turanica* have been reported in the studied areas, the north Iran (Figure 1). *Leishmania tropica* and *L. major* species are dominant in Golestan Province, while, *L. infantum* and *L. major* species prevail in Mazandaran Province. *Leishmania* infection has not been reported yet in human samples or possible reservoirs in Gilan (Guilan) Province and the west of Mazandaran until the time of compiling this research.

Table 1. Sampling locality, geographical coordinates, captured species, and their number at each region of the small mammals of the north of Iran, 2019–2023. Abbreviation: N: number; Lat: Latitude; Long: Longitude.

Province	Town	Sampling Locality	Num. of locality on the Fig. 1	Species	N	Lat.	Long.
Golestan	HosseinAbad	Kalpush	1	*Myotis blythii*	5	37.18	55.75
	Azad Shahr	Fazel Abad	2	*Lepus tibetanus*	2	37.06	55.20
	KhanBebin	Shirābād	3	*Rattus norvegicus*	3	36.98	54.99
				Mus musculus	1		
				Mustela nivalis	1		
	Inche Bouron	Tangeli	4	*Mus musculus*	3	37.45	54.71
		Ajigol	5	*Meriones libycus*	1	37.41	54.55
		Ashk Tappeh	6	*Meriones libycus*	3	37.43	54.60
				Rhombomys opimus	6		
		Alagol Lake	7	*Rhombomys opimus*	22	37.42	54.62
		Inche Bouron	8	*Microtus* sp.	1	37.46	54.72
	Aq Qala	Ghorban Abad	9	*Nesokia indica*	1	36.95	54.44
				Mus musculus	1		
		Agh Ghala	10	*Hemiechinus auritus*	1	37.04	54.46
		Mohammad Abad	11	*Nesokia indica*	1	36.91	54.42
				Rattus norvegicus	1		
		Saqar Tappeh	12	*Mus musculus*	17	37.17	54.48
				Microtus sp.	8		
		Torkaman Sahra	13	*Lepus tibetanus*	7	37.09	54.19
	Gorgan	Mohammad Abad	14	*Rattus norvegicus*	1	36.94	54.42
				Mus musculus	6		
		City Center	15	*Rattus norvegicus*	1	36.84	54.44
		Marzan Kala	16	*Rattus norvegicus*	2	36.81	54.56
		Gharn Abad	17	*Rattus norvegicus*	1	36.79	54.62
				Apodemus uralensis	2		
				Crocidura suaveolens	8		
		Tuskestan	18	*Rattus norvegicus*	7	36.79	54.57
				Apodemus uralensis	1		
				Apodemus hyrcanicus	1		
		Shast Kola	19	*Mus musculus*	1	36.83	54.35
				Hemiechinus auritus	1		
		Chale Bagh Park	20	*Pipistrellus kuhlii*	1	36.83	54.43
Mazandaran	Sari	Kiasar	21	*Apodemus witherbyii*	1	36.17	53.71
				Ochotona rufescens	1		
		Darab kola	22	*Crocidura* sp.	1	36.57	53.23
		Dashte Naz	23	*Erinaceus concolor*	1	36.82	53.17
		Valuja	24	*Rattus norvegicus*	2	36.79	53.20
		Chemaz Tappeh	25	*Mus musculus*	1	36.79	53.18
		Shahrak Farhangian	26	*Rattus norvegicus*	1	36.48	52.94
				Mus musculus	1		
		Qajar Kheyl	27	*Erinaceus concolor*	1	36.78	53.02
		Amreh	28	*Pipistrellus pipistrellus*	2	36.42	53.11
				Rhinolophus hipposideros	1		
	GhaemShahr	Siah Rood River	29	*Rattus norvegicus*	1	36.45	52.88

		Parchinak	30	*Rattus rattus*	1	36.45	53.01
		Eslam abad	31	*Rattus rattus*	1	36.46	52.88
				Crocidura caspica	1		
		Gharakheil	32	*Mus musculus*	4	36.46	52.77
		Vosta Kola	33	*Suncus etruscus*	5	36.37	52.50
				Crocidura sp.	6		
		Siahkola	34	*Apodemus uralensis*	1	36.44	52.85
		Ghadi Kola	35	*Glis persicus*	1	36.40	52.92
				Crocidura sp.	3		
		Berenjestanak	36	*Hystrix indica*	1	36.36	52.93
				Erinaceus concolor	1		
		Savadkooh	37	*Apodemus witherbyii*	2	35.96	52.67
	Babol	Moqri Kala	38	*Rattus rattus*	1	36.37	52.60
		Babol Rood	39	*Rattus rattus*	1	36.37	52.72
				Apodemus witherbyii	1		
		Muziraj	40	*Mus musculus*	1	36.54	52.64
	Amol	Dabudasht	41	*Rattus norvegicus*	1	36.48	52.45
		City Center	42	*Rattus norvegicus*	1	36.49	52.36
				Apodemus witherbyii	1		
		Larijan	43	*Apodemus witherbyii*	3	35.87	52.21
				Calomyscus. grandis	1		
		Malār	44	*Ochotona rufescens*	9	35.92	52.18
	Mahmud Abad	Tileksar	45	*Mus musculus*	1	36.61	52.26
	Chamestan	Kashpel park	46	*Rattus norvegicus*	1	36.48	52.11
				Apodemus witherbyii	2		
	Nur	Shahrak Vahedi	47	*Rattus norvegicus*	3	36.55	51.99
		Louzan park	48	*Rattus norvegicus*	1	36.56	52.02
				Apodemus witherbyii	2		
		Baladeh	49	*Ochotona rufescens*	13	36.18	51.78
	Royan	Shahrak Pasargad	50	*Rattus norvegicus*	1	36.53	51.99
				Suncus etruscus	2		
		Lale Royan Park	51	*Rattus norvegicus*	2	36.56	51.96
	Chalandar	Molkar	52	*Mus musculus*	1	36.58	51.68
	Shirud	Gil Mahale	53	*Apodemus witherbyii*	3	36.84	50.79
	Noshahr	Shahr-e-Posht	54	*Rattus norvegicus*	3	36.62	51.50
		Musa Abad	55	*Suncus etruscus*	1	36.62	51.50
		Darzikola	56	*Erinaceus concolor*	1	36.59	51.52
	Chaloos	Makarud	57	*Rattus norvegicus*	7	36.34	51.25
	Tonekabon	2 Hezar	58	*Rattus norvegicus*	2	36.64	50.76
		Qaleh Gardan	59	*Rattus rattus*	3	36.75	50.83
		Lashgarak	60	*Apodemus witherbyii*	2	36.76	50.89
				Crocidura caspica	1		
	Ramsar	Tosasan	61	*Rattus norvegicus*	2	36.92	50.64
Gilan	Masal	Shalma	62	*Rattus norvegicus*	2	37.30	49.03
		Owlam	63	*Rattus norvegicus*	2	37.45	49.09
				Rattus rattus,	7		
				Mus musculus,	1		
				Glis persicus	2		
				Rhinolophus hipposideros	3		
		Lowheh Sara	64	*Mus musculus*	2	37.36	49.13
		Duleh Malal	65	*Crocidura caspica*	6	37.36	49.15
		Shanbeh Rah	66	*Microtus obscurus*	33	37.29	48.93
		Sang Bast	67	*Crocidura caspica*	1	37.35	49.14
	Navrud	Vardeh Sara	68	*Mustela nivalis*	1	37.69	48.98

Masal	Merian	69	*Mus musculus*	1	37.85	48.62

Table 2. Ecological habitats of recorded and identified small mammals in the margins of the Caspian Sea, north of Iran.

Family	Species	Collection habitats				
		Plain areas	**Urban and Rural areas**	**Forest areas**	**Mountainous areas**	**Fields and gardens**
Muridae	*N. indica*	×	×			×
	R. norvegicus	×	×	×	×	×
	R. rattus	×	×	×		×
	M. musculus	×	×	×	×	×
	A. witherbyii	×		×	×	×
	A. uralensis			×		
	A. hyrcanicus			×		
	M. libycus	×				×
	R. opimus	×				×
Cricetidae	*M. obscurus*			×	×	×
Gliridae	*G. persicus*			×		×
Calomyscidae	*C. grandis*				×	
Hystricidae	*H. indica*			×		×
Soricidae	*S. etruscus*	×		×		×
	C. caspica	×		×		×
	C. suaveolens	×		×		×
	Crocidura sp.	×		×		×
Erinaceidae	*E. concolor*	×		×		×
	H. auritus	×				×
Leporidae	*L. tibetanus*	×				×
Ochotonidae	*O. rufescens*	×			×	
Vespertilionidae	*P. pipistrellus*			×		
	P. kuhlii		×	×		
	M. blythii			×		
Rhinolophidae	*R. hipposideros*			×		
Mustelidae	*M. nivalis*	×	×	×	×	×

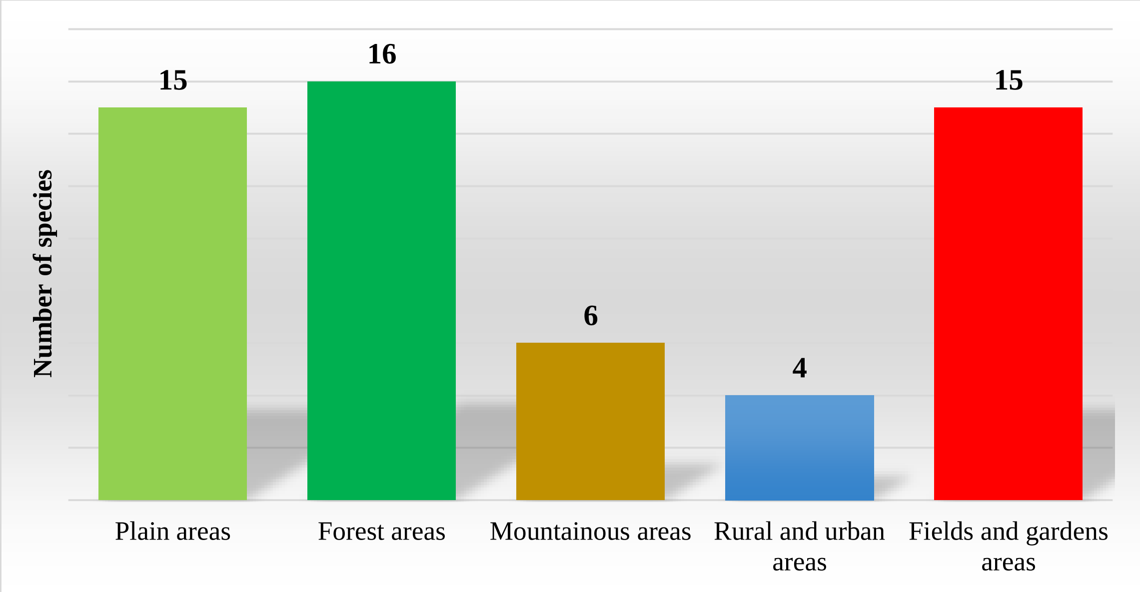

Fig. 2. Diagram of diversity of the captured species in the five ecological habitats.

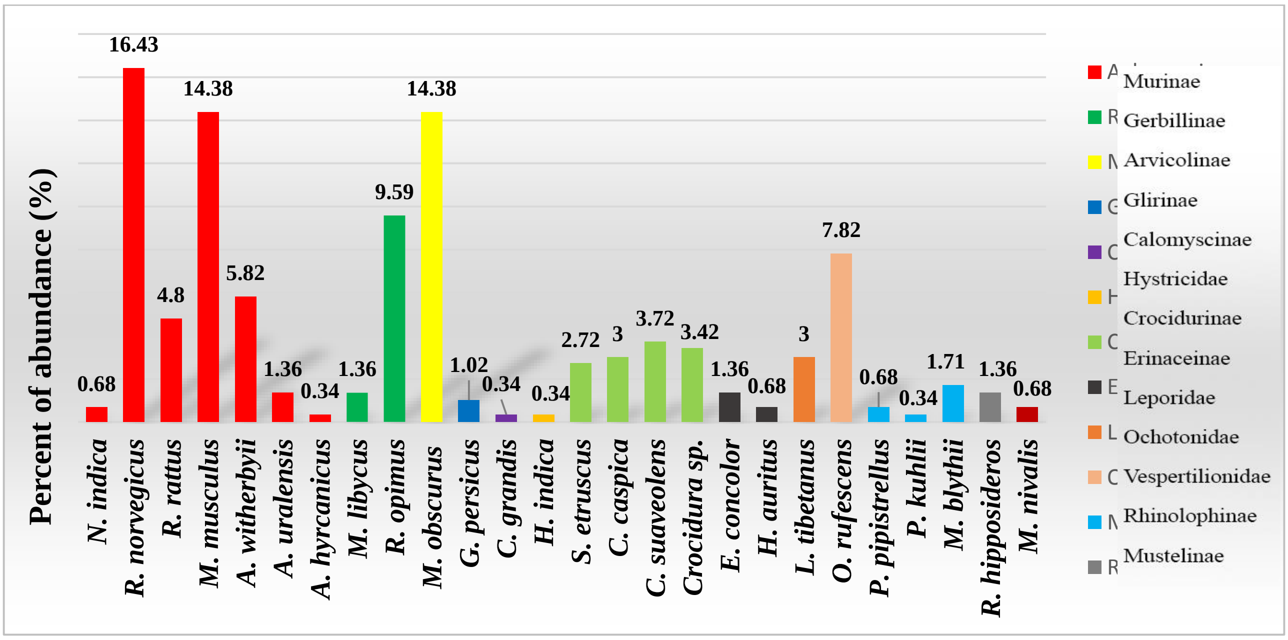

Fig. 3. Diagram of the abundance of captured samples per species (%), which are divided by different colors according to family and subfamily.

Discussion

The survey of diversity, abundance, and distribution of terrestrial small mammals is vitally important to manage and control vector-borne diseases. Climate changes and anthropogenic activities are drivers for increasing vector-borne diseases worldwide. Nowadays, there are concerns about altering vector host-pathogen relationships by these factors (21). There is an increased threat toward latitudes which are more affected by climate warming. Temperate regions are supposed to be the target of climate change effects on the relationship between biotic factors causing diseases (22, 23). A rise in vector-borne diseases such as Leishmaniasis associated with small mammal reservoirs was documented in some studies. Carnivora, Rodentia, and Eulipotyphla (previously known as insectivores) are the most small mammals being infected with *Leishmania* species (7, 9, 18, 24). Syanthropic rodents and the vicinity of rodent burrows in urban and rural areas can facilitate the transmission of CL to humans (25).

Table 3. The number of infection, percentage, and CI 95% infection species of the small mammals recognized as *Leishmania* sp. reservoirs in north Iran from 2013 to 2023. (T.N: Total number of investigated specimens for each species, N.I: Number of infected samples)

Species	T.N	N.I	Percent of infection	$CI_{95\%}$		P-Value
				Lower limit	Upper limit	
Rhombomys opimus	805	378	46.96%	40.56%	52.20%	0.021
Meriones lybicus	134	50	37.31%	31.01%	41.12%	
Mus musculus	95	13	13.68%	10.68%	16.00%	
Rattus norvegicus	16	3	18.75%	11.70%	25.75%	
Hemiechinus auratus	6	2	33.33%	27.23%	38.98%	
Rattus rattus	-	-	-	-	-	-
Nesokia indica	-	-	-	-	-	-
Meriones persicus	-	-	-	-	-	-
Total	1056	446	-	-	-	-

Since Iran is part of the geographical distribution of leishmaniasis many studies concentrated on the disease vectors and hosts in the different parts of the country. One systematic review and Meta-analysis reported Isfahan (66%) and Golestan (64%) Provinces as regions with the highest prevalence of CL associated with *L. major* while the lowest prevalence of CL was recorded from Kermanshah (4%), Hormozgan (10%), and Bushehr (12%) (26). In the spatial modeling of CL, from 1983 to 2013 represented by Holakouie-Naieni et al (27). Dry and desert climates in Iran used to be the endemic foci of the disease while the southwest of Iran was affected between 1983 and 1997, and subsequently, CL developed towards the center and eastern Iran between 1998 and 2013. Yazd, Khuzistan, and Kohgiloyeh-Boyer-Ahmad have also been supposed to be the hotspots for the disease. The results of both surveys and also present study are exactly fitted to the ecological niche modeling for the main reservoirs of CL (28). It is also noteworthy to mention that the occurrence of *Leishmania* in the north of Iran is mostly related to the presence of *R. opimus* and *N. indica* but in the central, south, and the western part of Iran, it is more associated with the occurrence of the genus *Meriones*, *Tatera* and also *Rhombomys*. The amastigotes of *Leishmania* parasite have been also observed in *M. musculus* and *R. rattus* in south Iran (29, 30) and *M. persicus*, *T. indica*, and *M. musculus* were PCR-positive rodents for *Leishmania major* DNA (30, 31).

In general, the highest *Leishmania* infection rate has been reported from members of subfamilies Gerbilinae and Murinae as main reservoir hosts of Zoonotic Cutaneous Leishmaniasis (ZCL) in North Iran (7). Synanthropic rodents infected with *Leishmania* species were found in 20 percent of Iran, aslo *N. indica* and *R. opimus* show the highest prevalence with 48% and 39% infection, respectively (32). Rejali et al. in their meta-analysis demonstrated that Forest plots of CL prevalence (95% CI per 100,000 population) were 45.6 (44.1–47.2) (33). Moreover, there have been trends of increase in the incidence of CL in Iran from 2019 to 2022 (34). However, at the same time, it seems like the distribution of CL has been extended to a new area. In contrast, VL is mainly endemic in restricted regions of Iran, notably the northwest (Ardabil Province) and southwest (Fars Province) (35). Cutaneous Leishmaniasis is increasing in Iran especially in the Golestan Province due to the extended distribution range of species from the subfamily Gerbilinae at endemic areas of ZCL (increasing animal reservoirs in a spatial and temporal) (36). For example, the distribution ranges of *R. opimus* and *M. libycus* have recently extended in the Caspian Sea lowland because of climatic changes and anthropogenic activities (37), which coincides with the increasing abundance of sand flies as well as *Leishmania* species in this area (26). As demonstrated in this study due to the presence of food and shelters, fields, gardens, and plain areas are much more occupied by potential reservoirs of *Leishmania*. In addition to climate change which may increase the length of suitable season for sandfly activities, the retreat of the Caspian Sea and left wetlands behind may increase the optimal area for sandfly spawning which was proposed to be more investigated. Moreover, deforestation and alteration of wild habitats have increased vector-host interaction and the risk of transmission of parasites to humans. Despite accessibility to food in the mentioned areas, lack of safety causes a rise in the population of rodents and other small mammals in urban and rural areas. On the other hand, the harsh climate of mountainous ranges and food shortages especially in cold seasons which are determinants for the living of small animals may cause low numbers of small mammals in mountains and their migration to urban and rural residential areas. Although bats have been reported as reservoirs of *Leishmania* throughout the world (38-40), there has not been a report of transmitting *Leishmania* into humans and livestock by bats in Iran yet. So the role of bats in the life cycle of *Leishmaina* should be investigated in Iran. Natural infection of Hedgehogs to *L. major* has been previously reported (41). Hedgehogs have been also reported as reservoirs of CL in Iran. The result of the survey conducted by Yaghoobi-Ershadi et al. demonstrated that the rate of small mammals infection was high in *R. opimus* (82.1%), then *M. libycus* (15.7%), and last *H. auritis* (2.2%) in specimens collected from Isfahan (42). The role of sand flies (Family: Phlebotominae) in the transmission of *Leishmania* is known (24). Several species of sand flies are distributed in the north of Iran some of these are known as the vectors of *Leishmania* like *Ph. papatasi*, and *Ph. Sergenti* (43-45). This issue can cause more transmission of *Leishmania* from the reservoirs to humans. According to the research done, it is suggested to identify new reservoirs, especially in the western areas of the northern strip of Iran. It is noteworthy to mention that stray dogs and other carnivores have been also reported as one of the main hosts for transmitting Leishmania parasites in vicinity to human.

Last but not least, during preparing the paper *Aedes* mosquitos and the viral infection of Dengue fever has been reported from different parts of Iran in 2024 (https://www.who.int/). *Aedes aegypti* and *Aedes albopictus*, the main vector for the Dengue virus may have also been coinfected with *Leishmania* which has to be more investigated in Iran (46).

Conclusion

Although there have been many studies on *Leishmania* reservoirs and vectors in Iran there were no comprehensive studies available to elucidate the role of each species of small mammals in spreading different kinds of *Leishmania* sp. Moreover, the importance of different small mammal ectoparasites as a vector of *Leishmania* is still vague which can be elucidated by applying a modern molecular approach. It is also proposed that the role of stray dogs in spreading leishmaniasis be studied. Additionally, a general survey to follow the effect of climate and ecological changes on the pattern of the leishmaniasis occurrence map is necessary. Mapping vector-reservoir ecology and disease incidence using upgraded modeling software is also recommended.

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Ethical Approval

This study was conducted by the Declaration of Helsinki. Approval was granted by the ethical committee of Golestan University of Medical Sciences, Gorgan, Iran with ethics code, IR.GOUMS.REC.1399.072.

Conflict of Interest

There is no conflict of interest.

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