Original Article

Pulex irritans on Dogs and Cats: Morphological and Molecular Approach

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Abstract

Background: The painful bite of *Pulex irritans*; causes wound on the host body and is a vector for *Bartonella* bacteria species, which can cause trench fever, *Rickettsia* species, Rocky Mountain spotted fever and Mediterranean spotted fever. We conducted a study to find out the hosts, abundance, as well as the molecular characteristics of this flea species in Meshkin-Shahr County, Ardabil Province, northwest of Iran.

Methods: After collecting fleas from different reservoir hosts and transferring them to the laboratory, identification was done using different morphological characters as well as the internal transcribed spacer1 (ITS1) molecular marker.

Results: This morphological study indicated that from the 1053 fleas, which were collected from 162 different animals, including cats and dogs, 74 specimens belonged to human flea, *Pulex irritans*. In addition, in molecular analysis showed a high sequence similarity (99.5%) with the *P. irritans* counterparts from Spain country and Zanjan of Iran available in GenBank.

Conclusion: *Pulex irritans* species is an obligatory hematophagous ectoparasite of human and animals. Therefore, considering the relatively high frequency of this species on the body of cats and dogs, it is suggested to conduct more studies on its distribution and the possibility of being a vector of pathogens among these animals and human. The results of these studies will be used to compile and review the control programs of this vector.

Keywords: *Pulex irritans*; Dog; Cat; ITS1; Iran

Introduction

Fleas, the common name for the order Siphonaptera, have unique properties like the lack of wings, sucking mouthparts, with a specific digestive system. Their digestive system is different from other blood-feeding insects structurally and functionally. The flea body pressed laterally. It is different from other insects that have stretched bodies from each side. In addition, a flea has a great leaping ability, which is the result of strong feet and the evolution of the curves of sides. It feeds from a wide range of hosts including birds and mammals. These features make this order of insect different from others (1). The results from the molecular studies on DNA and morphological studies and ovary features showed that the closest relative of fleas is Boreidae from Mecoptera species (2). Today in the world, over 2500 species of fleas have been identified whose hosts are mostly birds and mammals (3). The desire for blood feeding from warm-blooded animals has led to some of these species being vectors of some infectious diseases, and besides being the main host for some tapeworms like *Dipylidium caninum* (4). The family Pulicidae of fleas has a great desire to feed blood from humans or other mammals. The existence of similar morphological characters among different species of

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Pulex, as caused most of the species of this type, to be hard to differentiate (5). One of the most famous species of this genus is *Pulex irritans*. Despite its name, this species does not have a definite host and can suck blood from a wide range of mammals, and this factor makes it a carrier of various diseases such as *Dipylidium caninum* and trench fever. *Rickettsia* species, which can cause Rocky Mountain spotted fever and Mediterranean Spotted Fever (6). The existence of a wide range of hosts and compatibility with different climatic areas results in finding this flea in many different areas of the world (7). Most of the studies done on this species in some areas of Iran have related to morphological characters and there is little data about its molecular characters. In this study, the internal transcribed spacer1 of ribosomal DNA (ITS1-rDNA) gene, was determined to provide a molecular marker for identification of the local Iranian *P. irritans* population.

Materials and Methods

Study area

Ardabil Province is one of the thirty-one provinces of Iran. Meshkin-Shahr County is one of the counties located in the Province in the northwest part of Iran (Fig. 1). Meshkin-Shahr County with geographical coordinates 38° 44' N and 47° 40' E is at an altitude of 1400 meters above sea level.

Morphological studies

The present study was conducted during March and July 2018 in Meshkinshahr County, Ardabil Province. The sampling was done from random captured cats (*Felis catus*) and dogs (*Canis lupus*) specimens, with two standard methods of combing and separating with forceps (9). After collecting and recording the reservoir's host data; their ectoparasites were transferred to the glass tubes with lids containing 70% ethanol for further identification (Fig. 2). First, the flea specimens from each host were cleared with 10% KOH individually and the permanent slides in Canada balsam were prepared. For the morphological identification of flea specimens, we used the standard key of Harimalala et al. (10). After morphological identification and final confirmation, specimens were taken out of slides, washed, and transferred to tubes containing 90% ethanol.

Molecular Studies

The identified flea specimens were used for DNA extraction and molecular study. After morphological studies, a sample of each flea was placed under a binocular on a slide and its abdominal contents were completely emptied and its information was recorded and transferred to a new microtube. The flea was then crushed into a microtube with a pistol. The microtube was transferred to a nitrogen tank for 5 minutes and then the DNA of fleas was extracted using DNeasy kit (Qiagen, Hilden, Germany) and stored at -20 ºC for later use. The ITS1 primers used of *P. irritans* were NC5 (5′-GTA GGT GAA CCT GCG GAA GGA TCA TT-3′) and ITS1rev (5′-GCT GCG TTC TTC ATC GAC CC-3′) as forward and reverse respectively (11). In each 15µl reaction of PCR, the 0.50–1µl of each extracted DNA, 2µl primer (10pmol of each), 7.5µl 2x master mix (Taq DNA Polymerase Master Mix RED, 2mM $MgCl_2$, Amplicon) were used. The PCR reaction was conducted with a thermal cycler device along 33 cycles (94 degrees for 30 seconds, 58 degrees for 30 seconds, and 72 degrees for 1 minute) and for the final extension, 10 minutes with 72 degrees was used. The PCR products were visualized in a 1.5% agarose gel containing a DNA-safe stain (cat no., EP5082, Sinaclon). PCR product of each sample sequenced by the ABI3730XL sequence analyzer (Macrogen, S. Korea). The sequences were edited and aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and compared with reference sequences from GenBank. For ITS1 sequences, the phylogenetic tree was built with the maximum likelihood (ML) algorithm using molecular evolutionary genetics analysis (MEGA) software, in-

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cluding sequences representative of three families (Pulicidae, Leptopsyllidae, Ceratophylidae) of order Siphonaptera.

Results

The results of the morphological study indicated that among the 1053 flea specimens, which were isolated from two animal hosts, 47 dogs and 115 cats, 74 (7.03%) specimens belonged to *P. irritans*. It was specified that from 74 specimens of *P. irritans* fleas, 35 (47.29%) were males and 39 (52.70%) were females (Table 1). Out of the 74 *P. irritans* fleas, 49 (66.21 %) were collected from dogs; 32 (43.24%) were collected from female dogs, and 17 from male dogs, and the other 25 samples were taken from male cats. In this study, *P. irritans* flea were not found on female cats (Table 2). The morphological characteristic of *P. irritans* is shown in Figure 3.

The specific primers used in this study amplified about 900bp of ITS1 region of rDNA gene. (Fig. 4). The analysis of the ITS1 sequence of the identified isolates approved the morphological identification. The sequence of the isolated from the present study is accessible under GenBank Accession No. MG745174. After trimming the sequence data, 802bp of sequences were blasted. The sequence obtained in this study displayed 99.5% sequence homology with two *P. irritans* specimens from Spain (GenBank ID: LT797452) and Zanjan (GenBank ID: KX822017) in west of Iran (Fig. 5). In addition to these two sequences, there were some other *P. irritans* ITS1 sequences in GenBank originated from Spain, different locations of west of Iran (including Mahneshan Zanjan, Khoramabad, Kamyaran, Hamedan, Bahar, GilanGharb, Kerrmanshah, Mahabad, Sanandaj, and Urmia), China, and India which displayed 95.69-99.38% sequence homology with the sequence obtained in this study. To assess phylogenetic relationship among *P. irritans* isolates, multiple alignments were generated for a subset of *P. irritans* specimens plus the sequence obtained in this study, and the alignment was used to construct a phylogenetic tree by Neighbor Joining (NJ) method. Phylogenetic relationships of the *P. irritants* using the ITS1 region of the rRNA gene produced the highest log likelihood as shown in Figure 6. The sequence obtained in this study was strongly associated with the specimen from Spain (GenBank ID: LT797452). Most of the nodes in the tree had 98-99% bootstrap values indicating very strong support for the nodes created in the tree.

Table 1. Geographical features of the study area, prevalence and distribution of fleas and their animal hosts in Meshkinshahr County, Ardabil Province, Iran, 2016-2018.

Sampling Area	Location		dogs		cats		collected fleas	
	North	East	N	%	N	%	N	%
Parikhan	382452	473852	5	10.64	19	16.52	152	14.43
Meshkinshahr	382246	474054	7	14.89	25	21.79	101	9.59
Sarikhanloo	383105	473110	3	6.37	18	15.65	28	2.66
Urkandi	382115	473804	6	12.76	15	13.04	351	33.33
Ahmad Bigloo	382441	473336	3	6.37	8	6.96	187	17.76
Aghbalagh	382102	474023	6	12.76	6	5.22	6	0.57
Koojangh	382918	473053	8	17.02	19	16.52	204	19.37
Ahmad Abad	382200	473534	9	19.14	5	4.35	24	2.28
Total	……..	……	47	99.95	115	100	1053	99.99

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Table 2. Prevalence of host's flea infestation in Meshkinshahr County, Ardabil Province, Iran, 2016-2018. M: male flea, F: female flea

Sampling Area	Total Fleas	Fleas on Dogs				Fleas on Cats				*Pulex* No. (%)					
		No	%	*Pulex*	%/74	No	%	*Pulex*	%/74	Total	%	M	%	F	%
Parikhan	152	134	14.2	11	14.8	18	15.9	3	4.0	14	18.9	6	8.1	9	12.1
Meshkinshahr	101	79	8.5	1	1.3	22	19.4	1	1.3	2	2.7	0	0.0	2	2.7
Sarikhanloo	28	28	2.9	5	6.7	0	0.0	0	0.0	5	6.7	0	0.0	5	6.7
Urkandi	351	313	33.3	31	41.8	38	33.6	6	8.1	37	50.0	12	16.2	25	33.7
Ahmad Bigloo	187	182	19.3	2	2.7	5	4.4	0	0.0	2	2.7	0	0.0	2	2.7
Aghbalagh	6	6	0.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0. 0	0	0.0
Koojangh	204	187	19.8	9	12.1	17	15.0	2	2.7	11	14.8	7	9.4	4	5.4
Ahmad Abad	24	11	1.1	0	0.0	13	11.5	2	2.70	2	2.7	1	1.3	1	1.3
Total	1053	94	100	60	81.0	113	100	14	18.9	74	100	26	35.1	48	64.8

Fig. 1. Map of area study Meshkinshahr County, Ardabil Province, Iran.

Fig. 2. Isolation and collection of fleas from a dog in Meshkinshahr, northwest of Iran using the standard method of combing and transferring them to tubes containing 70% ethanol (original)

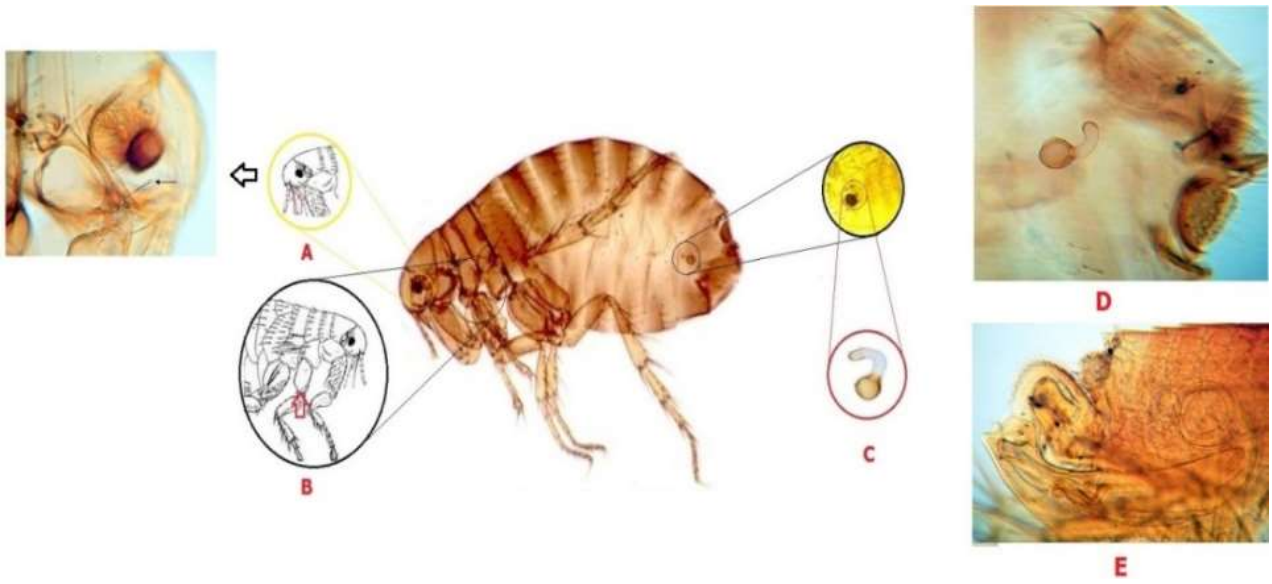

Fig. 3. Morphological characters of *Pulex irritans*. A: Eye hair is under the eye; B: The borderline of moral rood which does not exist; C: The first part of Spermatheca which is not distinguishing; D and E: end part of the female and male genus (original)

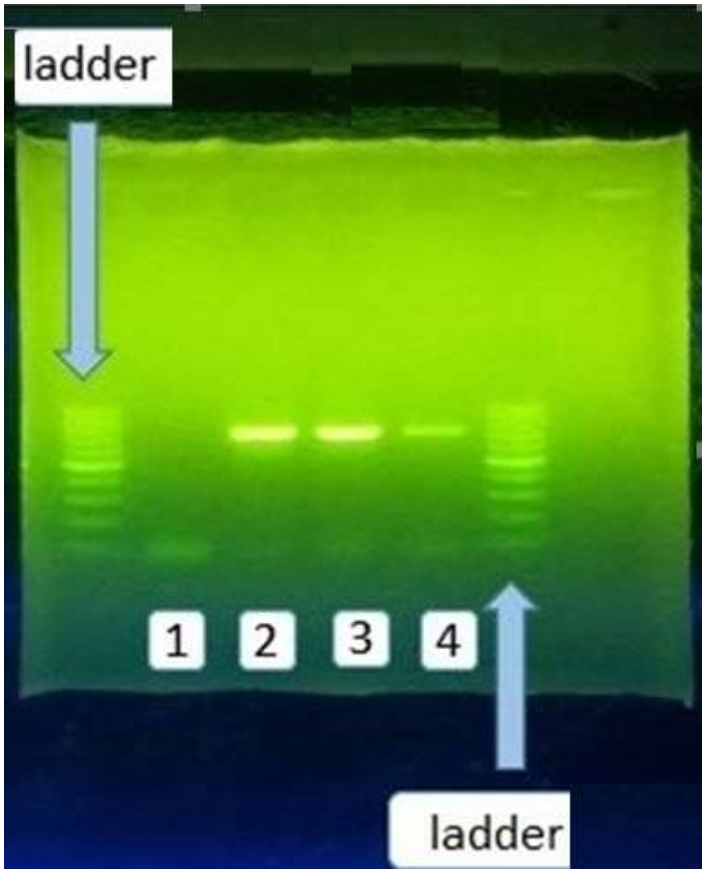

Fig. 4. The ITS1-rDNA PCR products (about 900bp) on agar gel 1.5%. Ladder: 100bp DNA Ladder (SinClon, Iran), 1: Negative control (ddH_2O); 2: *Pulex irritans* isolated from stray cats, 3 and 4: *Pulex irritans* isolated from stray dogs

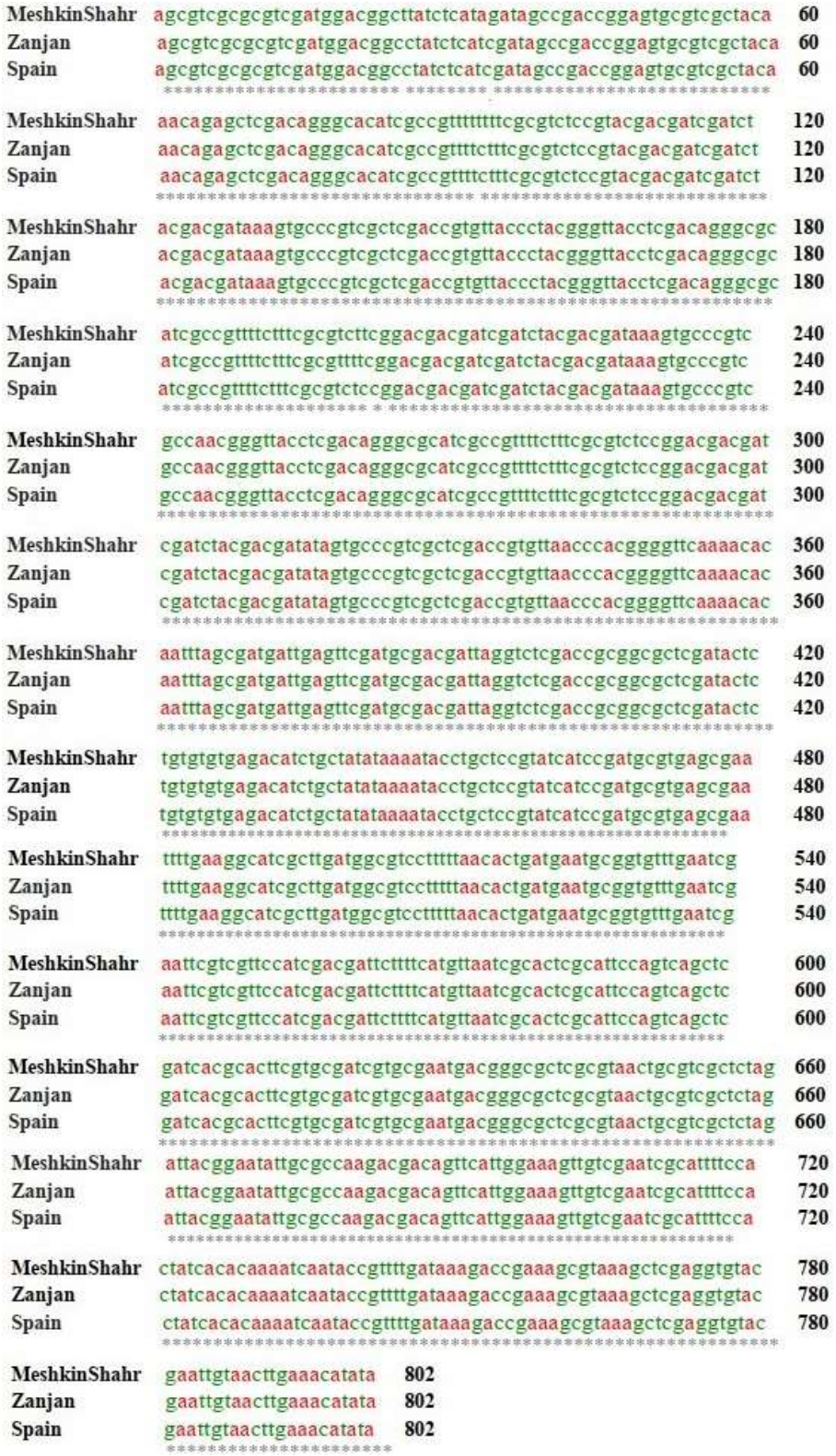

Fig. 5. The multiple alignments of 802bp of ITS1-rDNA sequence of *Pulex irritans* from this study (MeshkinShahr, GenBank ID: MG745174**)** and two reference sequences from Znajan, Iran (GenBank ID: KX822017) and Spain (GenBank ID: LT797452). There was 99.5% similarity between MeshkinShahr specimen with the Zanjan and Spain specimens. * indicates similar nucleotide, and gaps indicates substitution in the position

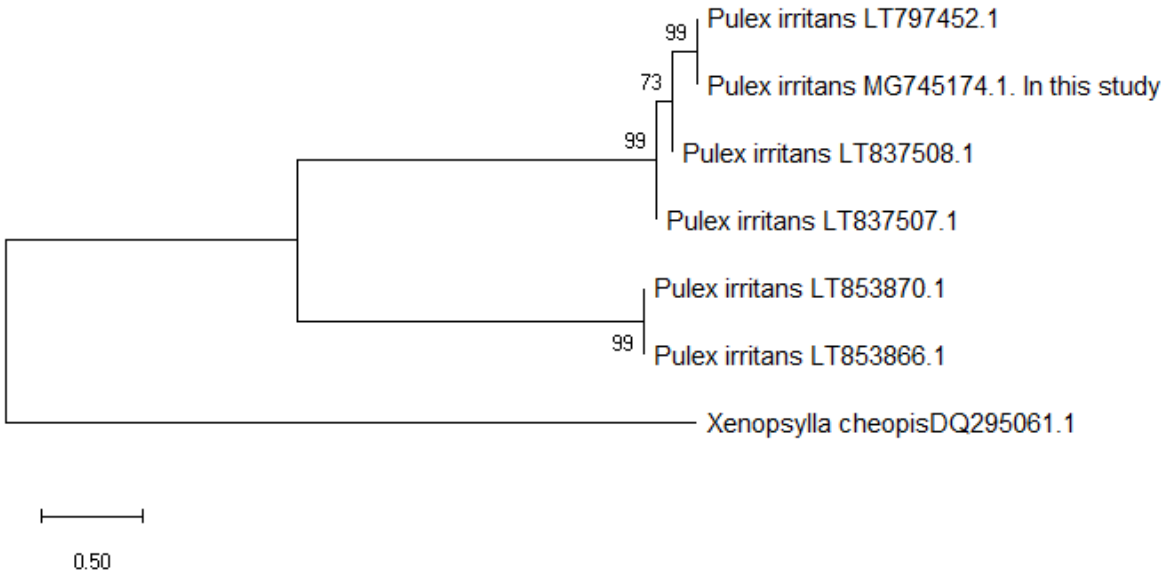

Fig. 6. Phylogenetic relationships inferred from neighbor joining analyses constructed by Mega10 based on 802bp sequences of ITS1-rDNA gene of *Pulex irritans* obtained in this study and a subset of *Pulex irritans* sequences available in GenBank. The GenBank ID of the sequences are shown in front of branches. *Xenopsylla cheopis* with accession number DQ 295061.1 was used as out-group. Scale bar indicates genetic distance. Our sample is marked with an arrow

Discussion

All human fleas collected specimens are characterized by laterally compressed bodies and large hind legs. *Pulex irritans* lack the combs that lend the appearance of genal and pronotal combs. It has a rounded forehead, which allows differentiation from the anteriorly flattened head of the stick-tight flea. Behind the antennae, the head has only a single pair of hairs. It has no pleural rod, a feature that differentiates it from *Xenopsylla cheopis* (12). In terms of medical importance, *P. irritans* can play the role of the intermediate host of the dog tapeworm (for example *D. caninum*). In addition, *P. irritans* may be a vector for erysipeloid (13). Moreover, the *P. irritans* is implicated in the spread of diseases historically associated with *X. cheopis* (14). The human female flea can lay more than 400 eggs in her lifetime. The ability of pupae and "cocoons" adults to lie dormant for a year or more can confound efforts at flea control. It does not kill adult fleas, but it does prevent reproduction. Adverse reactions flea bites appear as tense, pruritic, urticarial papules on exposed areas including the hands, forearms, and lower legs. Excoriation with secondary impetiginization is common. Vesicular and bullous flea bite reactions occur and can be quite dramatic. Histologic examination reveals a wedge-shaped dermal infiltrate that surrounds both venules and arterioles. Eosinophils are common. Epidermal necrosis, spongiosis, and intraepidermal or subepidermal bullae may be seen. The human flea is distributed throughout the world and has been recorded in a wide range of mammal hosts including humans, domestic dogs, cats, pigs, badgers (*Meles meles*) and foxes (*Vulpes vulpes*) (15). This species is also medically important as a possible vector of flea-borne diseases such as tularemia, murine typhus, and tapeworm (16).

In different parts of the world, for isolating and identifying *P. irritans*, different characters have been used. Out of the genus *Pulex*, the species *P. irritans* is the most abundant in the world and in Iran (17). The results of the morphological studies indicated that out of the

1053 fleas, 74 specimens belonged to *P. irritans*. In a similar study for investigating ectoparasite of dogs and cats in Kurdistan Province west of Iran and bordered with the northeast of Iraq country, this species was isolated with *Ctenocephalides felis* and *C. canis* but with a lower percentage of frequency (18). Moreover, in a review by Maleki-Ravassan et al. (19) on ectoparasite of dogs in different parts of Iran, *P. irritans* species and two species of *C. felis* and *C. canis* were isolated from hosts. In addition, another study, which was conducted on dogs in Shiraz, had similar results (20). In a study in 2015 for identifying ectoparasite on sheep in Kurdistan Province bordered with the east of Iraq country, 1323 specimens of *P. irritans* were isolated from hosts (21). In addition, Sharifdini et al. 2021 were collected *P. irritans* from seven raccoons from Guilan Province, northern Iran (22). In comprehensive research by Barutzki and Schaper (23) in 2000 for identifying ectoparasite of dogs and cats in Germany, out of 1508 cats and 2653 dogs, *C. felis*, *C. canis*, and *P. irritans* were isolated. Already, there are about 2500 species and subspecies of fleas in about 220 genera, but only relatively, few are important human pests. In this study, the result of the morphological analysis showed that the identified flea was *P. irritants*. The ITS1 gene sequence obtained in this study plus the phylogenetic tree, and high sequence homology with other available data in GenBank, confirm and support the result of our morphological study.

Conclusion

Pulex irritans is one of the most important ectoparasites of humans, which transmits various pathogens (including bartonellosis and rickettsioses) and causes skin sensitivity and irritation by biting humans and animals. This flea is transmitted from the cats and dogs body to humans and can bite humans. To achieve control of parasitic skin diseases of *P. irritans* in developing countries, local epidemiology has to be better understood. Studies in Iran on epidemiology and the medical significance of *P. irritans* in different areas of animals are limited. Molecular approaches, although in their infancy, are now providing a better understanding of the biology of ectoparasites and will become cornerstones for prevention and control in the future. Therefore, this study was conducted in order to accurately identify this vector based on morphological and molecular characteristics. Morphological studies show that there is no moral root border in this flea and the first part of the spermatheca is not differentiated. In this study, *P. irritans* was not caught in the body of female cats, which is not important from the point of view of disease epidemiology because both male and female fleas are able to feed on blood and transmit the disease to cats, dogs, and humans. The specific primers used in this study amplified the ITS1 regions of about 900bp. It is suggested that to find out the distribution of *P. irritans* in male and female cats, as well as the possibility of *P. irritans* being a vector among cats, dogs and human, more studies should be conducted at the country level.

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Ethical considerations

The protocols were conducted in this study followed the guidelines of the institutional ethical committee (Tarbiat Modares University). The protocols were approved by TMU ethical committee under registry TMU-3674.

Conflict of interest statement

Authors declare that there is no conflict of interest.

References

1. Zurita A, Callejón R, De Rojas M, Cutillas C (2019) Origin, evolution, phylogeny and taxonomy of *Pulex irritans*. Med Vet Entomol. 33(2): 296–311.
2. Perez-Martinez L, Venzal JM, Gonzalez-Acuna D, Portillo A, Blanco JR, Oteo JA (2009) *Bartonella rochalimae* and other *Bartonella* spp in fleas, Chile. Emerg Infect Dis. 15(7): 1150–1152.
3. Lareschi M, Venzal JM, Nava S, Mangold AJ, Portillo A, Palomar-Urbina AM, Oteo Revuelta JA (2018) La pulga humana *Pulex irritans* (Siphonaptera: Pulicidae) en el noroeste argentino, una investigación de *Bartonella* y *Rickettsia* spp. Rev Mex Biodivers. 89(2): 375–381.
4. Fontalvo MC, Favacho ARdM, Araujo AdC, Santos NMd, Oliveira GMBd, Aguiar DM, Lemos ERSd, Horta MC (2017) *Bartonella* species pathogenic for humans infect pets, free-ranging wild mammals and their ectoparasites in the Caatinga biome, Northeastern Brazil: a serological and molecular study. Braz J Infect Dis. 21(3): 290–296.
5. Foley P, Foley J, Sándor A, Ionică A, Matei I, D'Amico G, Gherman C, Domșa C, Mihalca A (2017) Diversity of flea (Siphonaptera) parasites on red foxes (*Vulpes vulpes*) in Romania. J Med Entomol. 54(5): 1243–1250.
6. Takahashi K, Takahashi M, Hamasaka K (2016) Finding of the human flea *Pulex irritans* (Siphonaptera: Pulicidae) in Hokkaido, Japan with human dermatitis caused by this flea. Med Entomol Zool. 67(4): 233–235.
7. Kumsa B, Abiy Y, Abunna F (2019) Ectoparasites infesting dogs and cats in Bishoftu, central Oromia, Ethiopia. Vet Parasitol Reg Stud Reports. 15: 100263.
8. Mohebali M, Malmasi A, Khodabakhsh M, Zarei Z, Akhoundi B, Hajjaran H, Azarm A (2017) Feline leishmaniosis due to *Leishmania infantum* in Northwest Iran: The role of cats in endemic areas of visceral leishmaniosis. Vet Parasitol Reg Stud Reports. 9: 13–16.
9. Hernández-Urbina CF, Vital-García C, Ávila AME, Colima AG, Sánchez-Olivas MP, Clemente-Sánchez F (2020) First report of Siphonaptera parasites in *Canis latrans* in the Flora and Fauna Protection Area, Médanos de Samalayuca Chihuahua, Mexico. Vet Parasitol Reg Stud Reports. 20: 100379.
10. Harimalala M, Ramihangihajason T, Harimanana R, Girod R, Duchemin JB (2021) Illustrated Morphological Keys for Fleas (Siphonaptera) in Madagascar. J Med Entomol. 58(4): 1701–1716.
11. Marrugal A, Callejón R, De Rojas M, Halajian A, Cutillas C (2013) Morphological, biometrical, and molecular characterization of Ctenocephalides felis and Ctenocephalides canis isolated from dogs from different geographical regions. Parasitol Res. 112(6): 2289–2298.
12. Darvishi MM, Youssefi MR, Changizi E, Lima RR, Rahimi MT (2014) A new flea from Iran. Asian Pac J Trop Dis. 4: 85–87.
13. Sanusi ID, Brown EB, Shepard TG, Grafton WD (1989) Tungiasis: report of one case and review of the 14 reported cases in the United States. J Am Acad Dermatol. 20: 941–944.
14. Hamzaoui EL, Zurita B, Cutillas A, Parola C (2020) Fleas and flea-borne diseases of North Africa. Acta Trop. 211: 105627.
15. Anstead GM (2020) History, rats, fleas, and opossums: the ascendency of flea-borne typhus in the United States, 1910–1944. Trop Med Infect Dis. 5(1): 37.
16. Bourne D, Craig M, Crittall J, Elsheikha H, Griffiths K, Keyte S, Merritt B, Richardson E, Stokes L, Whitfield V (2018) Fleas and flea-borne diseases: biology,

control and compliance. Companion Animal. 23(4): 204–211.
17. Seidy S, Tavassoli M, Malekifard F (2022) Pyrethroids resistance in Pulex irritans and *Ctenocephalides canis* in west and northwest Iran. Vet Res Forum. 13(4): 529–535.
18. Yakhchali M, Bahramnejad K (2015) A survey of *Pulex irritans* (Linnaeus 1758, Siphonaptera: Pulicidae) infestation in sheep and residential areas in Kurdistan Province, Iran. Iran J Vet Sci Technol. 7(1): 40–47.
19. Maleki-Ravasan N, Solhjouy-Fard S, Beaucournu JC, Laudisoit A, Mostafavi E (2017) The fleas (Siphonaptera) in Iran: diversity, host range, and medical importance. PLoS Negl Trop Dis. 11(1): e0005260.
20. Shoorijeh SJ, Ghasrodashti AR, Tamadon A, Moghaddar N, Behzadi MA (2008) Seasonal frequency of ectoparasite infestation in dogs from Shiraz, Southern Iran. Turk J Vet Anim Sci. 32(4): 309–313.
21. Tavassoli M, Ahmadi A, Imani A, Ahmadiara E, Javadi S, Hadian M (2010) Survey of flea infestation in dogs in different geographical regions of Iran. Korean J Parasitol. 48(2): 145–149.
22. Sharifdini M, Norouzi B, Azari-Hamidian S, Karamzadeh N (2021) The first record of ectoparasites of raccoons (*Procyon lotor*) (Carnivora, Procyonidae) in Iran. Persian J Acarol. 10: 41–54.
23. Barutzki D, Schaper R (2003) Endoparasites in dogs and cats in Germany 1999–2002. Parasitol Res. 90 Suppl 3: S148–150.