



# The first report of *Hoploleura sciuricola* infestation in a gray squirrel in Iran

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## Abstract

The popularity and maintenance of the Iranian gray squirrel have been increasing in the last decade. However, due to the special conditions of keeping and the exotic nature of this animal, the possibility of transmission of pathogenic objects such as bacterial, fungal, and parasitic infections and the transmission of many common diseases between humans and animals are raised in a case study of a collar infestation. The female squirrel was observed to be 65 days old and morphological examinations revealed the possibility of infestation with *Hoploleura sciuricola* lice. Previously, there were no reports of infestation with this louse in Iran. To accurately determine the sex and species of this louse, two pairs of primers designed from the mitochondrial gene (COX-I) that had protected regions in the genus of this louse were used, which led to the confirmation of the identity of the infestation and the first report of the existence of this louse in Iran was confirmed.

**Keywords:** Squirrel, Lice, Case Report, *Hoploleura sciuricola*

## Introduction

Persian gray squirrel with scientific name (*Sciurus anomalus*) is a rodent native to the western regions of the country. This species is found in the forest areas of Zagros from Sardasht of West Azerbaijan to Chaharmahal, Lorestan, Kohgiluyeh, and Fars and the habitat of this animal

include Iran, Armenia, Azerbaijan, Georgia, Greece, Iraq, Palestine, Jordan, Lebanon, Syria, and Turkey. The special type of nutrition, which is mainly from the fruits of oak and mountain pistachio trees, is very important. In the last decade, due to the prevalence of drought and reduced forest density in western Iran, as well as the prevalence of special and exotic animals Adults of these animals have flourished so much that unfortunately, we see the sale of infants of this animal in many illegal pet sales centers. Therefore, keeping this animal is due to the lack of special environmental and nutritional conditions, as well as bacterial, fungal, and parasitic infections, especially external skin parasites such as fleas, ticks, lice, and scabies, so the present study investigates a case of infestation. Dealing with *Hoplopleura sciuricola* lice on a gray-headed squirrel collar.

## Martial and methods

### Clinical description

Examination of the animal hematology (CBC) blood sample showed a relatively significant reduction in blood factors based on anemia and chronic infection of the animal (Table 1).

Table 1. The results of the squirrel hematology test studied

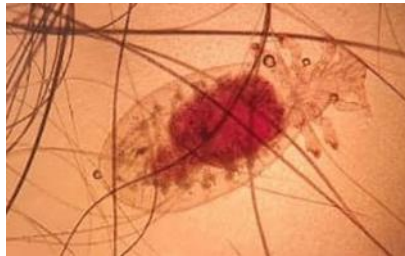
WBC (*10 <sup>9</sup> /l)	12.9	Net (%)	73
RBC (*10 <sup>12</sup> /l)	2.23	Lym (%)	19
HGB (g/dl)	8.60	Mon (%)	5
HCT (%)	31	Eos (%)	3

In the parasitological examination of the lice sample taken from the animal's body and using parasitology diagnostic keys, the blood-sucking lice *Hoplopleura sciuricola* from the family Hoplopleuridae was detected. Hemoploid flora is probably involved in the transmission of some pathogens such as tularemia, typhoid fever, and *Trypanosoma lewisi*. (Franchini *et al.* 2021; Mazharul Islam *et al.* 2021; Roth *et al.* 2018). Males are 900 microns in size and females are 1.5 mm. It is a small piece of lice that can be seen on the body when the infestation is severe. The head of the lice is round and the first band of the tentacle is wide and large, the third band in males has an appendage that causes its diagnosis. The chest is longer than the head and in the first pair of legs it looks a bit smaller than the other two pairs, the abdomen is oval and in the margin of the bands, it looks serrated, about 16 thorns of different sizes are seen on each band (Figure No. 2). There are airways on either side of it, the last ring carrying the genitals, and the

legs are relatively strong and end in strong claws (Taylor *et al.* 2007; Mehlhorn 2012). This lice can also be effective in transmitting mouse typhus from mouse to mouse (Foley *et al.* 2007).



**Figure 1.** A clinical picture of the squirrel studied



**Figure 2.** Lice sample is taken from squirrel study mode

To identify and determine the sex and species of the lice using molecular biology and using specific primers EU-1 and EU-2 with access number (EU375764) which reproduce a piece with a length of 378 bp used For DNA extraction was performed using MBST DNA extraction kit (Iran) and according to the manufacturer's instructions (Table 2). All extracted DNA was stored at  $-21^{\circ}\text{C}$  until use. To perform PCR reaction of primers E1 (Forward) and E2 (Reverse) that can amplify the mitochondrial genome of cytochrome oxidase one (CO-I) in *Hoplopleura sciuricola* lice. (Registered in the World Gene Bank) and was used to produce a PCR product with a length of 378 bp (Table and Figure 2). The total volume of PCR product is  $50\ \mu\text{l}$  and includes  $4\ \mu\text{l}$  Genomic DNA, One Time PCR Buffer,  $1.5\ \text{U}$  Taq Polymerase (Ampliqon, Denmark),  $30\ \text{pmol}$  of each primer (Metabion, Korea),  $100\ \mu\text{M}$  of each dATP, dTTP, dCTP, dGTP (Ampliqon, Denmark) and  $1.5\ \text{mM}$   $\text{MgCl}_2$ . An automatic thermocycler (SimpliAmp, USA) was used for the PCR of samples. PCR program for gene amplification including primary degradation at  $94^{\circ}\text{C}$  for 10 minutes, 40 cycles including degradation at  $94^{\circ}\text{C}$  for 30 seconds, primer binding at  $60^{\circ}\text{C}$  for 30 seconds, amplification at  $72^{\circ}\text{C}$  for 10 minutes 30 seconds, and finally  $72^{\circ}\text{C}$  for 5 minutes. Genomic DNA-free samples were used as negative control and PCR products were analyzed on 1.5% agarose gel in  $0.5 \times \text{TBE}$  buffer and stained using ethidium bromide and observed in a UV illuminator (Yi-Tian *et al.* 2020).

**Table 2.** Nucleotide sequences of primers for amplification of the CO-I region

Name of reactions	Name of Primer and accession no of the Corresponding gene	The nucleotide sequence (5' - 3')	PCR product
PCR for samples	EU-1	GAAGTGTATATTYTAATCCTTCCCGGATTT	378 bp
	EU-2	GATTAGTTCTGGCTAACTCTTCAATATATT	

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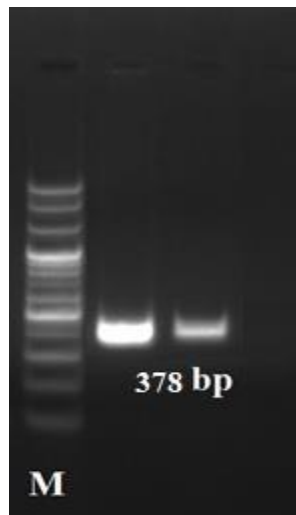
1  GAAGTGTATA TTYTAATCCT TCCCGGATTT GGACTTATCT CTCATATGAT TATAGAAGAG
61  TCTTCAAAG CTGAGGTGTT TGGCAGGTTA GGTATAATCT ATGCCATAGT GGCTATTGGT
121 GCCTTAGGGT TTGTAGTTTG GGCCACCAC ATATTCCTG TAGGTCTTGA CGTGGATAGC
181 CGGGCTTACT TTACAAGGGC CACTATAATT ATCGCTGICC CTACTGGTGT GAAGGTGTTT
241 AGATGAATTG CTACTTTATT CGGAGGGACT TCAGTAAAAG AAGTCTCCAT GCTTTGAAGA
301 TACGGGTTTA TTTTCCTATT TACAGTAGGC GGATTAACCG GATTAGTTCT GGCTAACTCT
361 TCAATGATA TTGTAATT

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Figure 3. Nucleotide sequence of the fragment for amplification of the CO-I region

## Results

The amplified fragment size (COX-I) after PCR reaction with the mentioned primers was 378 bp and the selected positive control sample could be amplified with the mentioned primer.

**Figure 4.** Electrophoresis results of PCR products

For treatment, first supportive treatment was performed to eliminate anemia, then by preparing a 0.5 per 1000 dilution of 35% Propetamphos insecticide toxin and using the pour-on method once every 3 days for 4 times to treat and remove lice from the animal's body. After complete treatment, we saw a significant

improvement in the excretion of lice from the body and hair of the animal, as well as a decrease in skin sensitivity to the presence of lice.

## Discussion

The first scientific report of *Hoplopleura sciuricola* was found in a squirrel native to Ireland in 1982. Later, there were several reports of rodent infestation in this lice in other parts of the world. This is even though the prevalence and extent of infestation with this lice have been present in almost all parts of the world, the research conducted is the first report of infestation of an Iranian gray squirrel with this type of lice (Shinozaki *et al.* 2004; Uslu *et al.* 2008). Due to the specific evolution of lice and the high severity of the epidemic and rapid transmission between other animals and the effects of severe to chronic anemia that can in acute cases cause the death of the animal or the transmission of pathogenic objects such as protozoa and bacteria. Cause pathogens in humans and other animals. Another important issue is not observing the quarantine of animals, especially when entering the breeding or maintenance complex, will increase the risk of contracting this parasite. Therefore, observing this issue will help in helping the public health of human beings as well as keeping and preserving endangered animals.

## Conclusion

The result of the present study shows the biological presence of *Hoplopleura sciuricola* louse and this research is the first report of the existence of this type of louse in Iran and considering the potential of transmission of common diseases between humans and animals and the hidden dimensions of some infectious diseases that can be transmitted from animals Exotic and wildlife to humans, more and additional studies should be done on such cases.

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