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Flea Species from Foxes: Identification via Microscopy and Molecular Techniques

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Abstract: *Fleas are ectoparasites of significant veterinary and public health importance, serving as vectors for various pathogens. This study aimed to identify flea species collected from red foxes (Vulpes vulpes) in Bihor County, Romania, using optical microscopy and molecular biology techniques. A total of 73 flea specimens were collected from hunted foxes and examined morphologically under a stereomicroscope. Taxonomic identification revealed four flea species: Pulex irritans (48 specimens), Ctenocephalides canis (18 specimens), Chaetopsylla trichosa (5 specimens) and Chaetopsylla globiceps (2 specimens). For molecular analysis, five representative flea specimens were selected, and DNA extraction was followed by polymerase chain reaction (PCR) amplification, targeting mitochondrial COX-2 gene region. Finally the results will be confirmed by sequencing methods. These findings underscore the necessity of ectoparasite surveillance in wild carnivores, considering their potential role as reservoirs for zoonotic pathogens. To minimize the risk of flea-borne disease transmission, preventive measures such as restricting contact between domestic and wild canids, implementing regular ectoparasite control in hunting dogs, and using protective equipment when handling fox carcasses are strongly recommended.*

• Introduction

Fleas are known as wingless insects and are obligatory ectoparasites that feed on blood. They belong to the order Siphonaptera, with the most important species of medical and veterinary significance classified within the families *Ceratophyllidae* (*Ceratophyllus gallinae, C. niger, Nosopsyllus fasciatus*) and *Pulicidae* (*Ctenocephalides canis, C. felis, Echidnophaga gallinacea, Pulex irritans, Spilopsyllus cuniculi, Tunga penetrans, Xenopsylla cheopis*).

Fleas parasitize a wide range of hosts, including wild canids and felids, and can subsequently be transmitted to domestic cats and dogs. In Europe, the increasing population of red foxes and ongoing urbanization contribute to a heightened risk of parasite transmission to domestic animals and, consequently, to humans. In this context, the red fox (*Vulpes vulpes*) plays a key role as a reservoir host for various pathogens, highlighting the importance of studying ectoparasitism in this species.

Fleas are capable of transmitting several pathogens, such as *Bartonella henselae* in the case of Ctenocephalides felis, or the cestode *Dipylidium caninum*. To date, only a limited number of studies have investigated flea infestation in wild canids, resulting in a lack of comprehensive data on flea prevalence. Most existing data pertain to fleas found on red foxes (*Vulpes vulpes*). Several studies conducted in Austria have reported *Chaetopsylla globiceps* as the most widespread species, identified in 10% to over 30% of the foxes examined.

The present study was undertaken to identify the flea species collected from the red fox (*Vulpes vulpes*) using both optical microscopy and molecular biology techniques.

• Material and method

A total of 73 flea specimens were collected from red fox (*Vulpes vulpes*) carcasses within the Bihor County region.The flea samples were examined under a light microscope for the identification of specific morphological characteristics. Following morphological identification, five distinct samples were selected for molecular analysis to detect parasite DNA. DNA extraction was performed using the BIOLINE® Tissue Protocol Kit (BIOLINE®), following the methodology described by Hornok et al. in a study conducted in 2018. Polymerase chain reaction (PCR) was employed to amplify the target DNA. The amplification process specifically targeted a ~780 bp fragment of the COX-2 gene, using the primers F-Leu (5'-TCT AAT ATG GCA GAT TAG TGC-3') and R-Lys (5'-GAG ACC AGT ACT TGC TTT CAG TCA TC-3').

A MyTaqTM Red Mix Master Mix (BIOLINE®) was used for the reaction. The amplification program was carried out with the My Cycler thermocycler (BioRad®). This program included the steps of DNA denaturation at 95°C for 1 minute; 40 cycles of: denaturation at 95°C for 30 seconds, hybridization at 53°C for 30 seconds and extension at 72°C for 30 seconds; followed by incubation at 4°C.

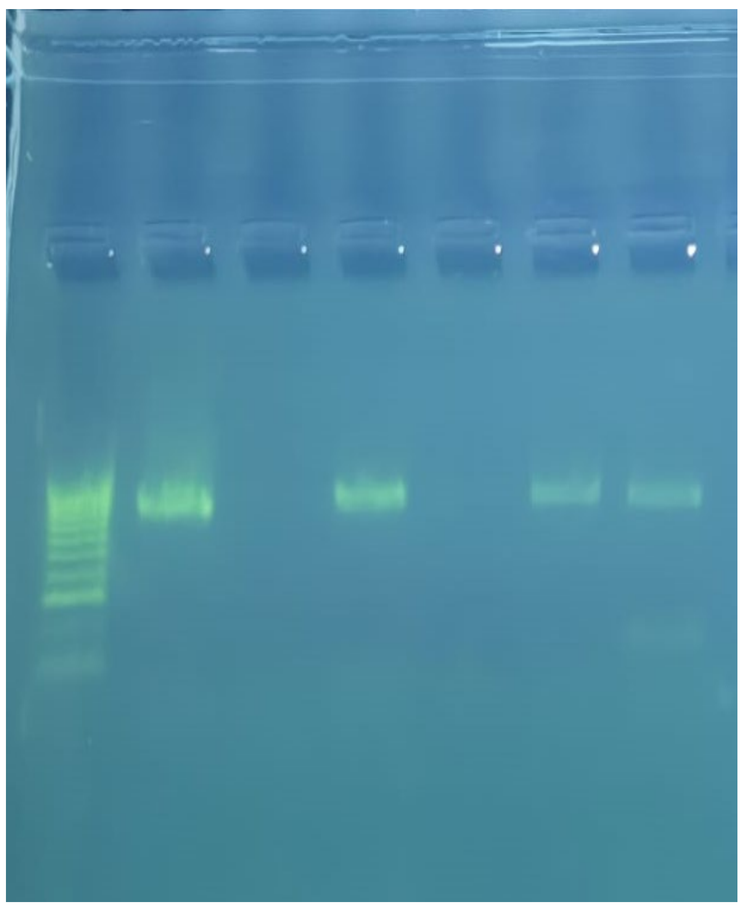
Amplicon analysis and control was performed by horizontal electrophoresis in a 1.5% agarose gel and images were captured with a digital camera.

Sample preparation for amplification and the use of the MyCycler Thermal Cycler (Bio-Rad®) equipment.

• Results and discussions

The identified flea specimens belonged to the following species: *Pulex irritans* – 48 samples (65.7%), *Ctenocephalides canis* – 18 samples (24.65%), *Chaetopsylla trichosa* – 5 samples (6.84%), and *Chaetopsylla globiceps* – 2 samples (2.73%). Following identification by PCR, it was found that 3 out of the 5 analyzed samples were successfully amplified. The amplified samples belonged to the following species: *Chaetopsylla globiceps, Chaetopsylla trichosa and Pulex irritans.* These samples will be subjected to sequencing for species confirmation.

Similar results were obtained in the study conducted by Hornok et al. in Hungary, where molecular biology techniques identified the species *Chaetopsylla globiceps* (3 samples) and *Ch. trichosa* (3 samples) exclusively on red foxes. Additionally, in a study conducted in Romania in 2017, alongside the two *Chaetopsylla* species, several other flea species were identified on red foxes, including: *Ctenocephalides canis* (32.6%), *Ctenocephalides felis* (0.1%), *Pulex irritans* (29.9%), *Paraceras melis* (3.2%), and *Ctenophthalmus assimilis* (0.1%).



• Conclusions

Optical microscopy analysis of the flea specimens collected from red foxes revealed the presence of the following species: *Pulex irritans, Ctenocephalides canis, Chaetopsylla trichosa*, and *Chaetopsylla globiceps.*

Three flea samples were successfully amplified and are to be submitted for sequencing in order to analyze the DNA and confirm the amplified species.