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Traumatic Myiasis in Wild Animals in Türkiye; First Report Myiasis Euphrates Soft-Shelled Turtle (*Rafetus euphraticus*) and Wild Goat (*Capra aegagrus*)

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Abstract: Traumatic myiasis is a parasitic infestation resulting from feeding fly larvae in traumatic lesions in the cutaneous tissues of vertebrate hosts. Species that cause traumatic myiasis are found in the families Calliphoridae and Sarcophagidae. This study evaluated the traumatic myiasis cases detected during examining animals brought to Dicle University Wildlife Rescue, Rehabilitation, Training, Practice, and Research Center with wound complaints between 2020-2022. Species determinations of the larvae collected from traumatic myiasis cases were detected in five different animals were made as morphological and molecular, and four species were detected, including *Lucilia sericata*, *Wohlfahrtia magnifica*, *Calliphora vicina*, and *Calliphora vomitria*. We report a case of traumatic myiasis in the Euphrates softshell turtle and Wild goat for the first time. In order to reduce the exposure of wild animals to traumatic myiasis, new strategies for fly control need to be developed.

Keywords: Euphrates soft-shelled turtle; traumatic myiasis; wild goat; wild birds; COI gene

1. Introduction

Myiasis, derived from the Greek word "myia" meaning fly, is defined as the infestation of Diptera larvae in vertebrate humans and animals for at least one period of their lives and spending their lives feeding on their living and dead tissues, liquid substances of their bodies and digested food [1]. Myiasis is divided into groups as cutaneous, nasopharyngeal, ocular, intestinal, or urogenital myiasis according to the affected body parts and tissues and as obligatory, facultative, and accidental myiasis according to the degree of parasitism displayed by the fly [1,2]. Traumatic myiasis, a type of cutaneous myiasis, is defined as a parasitic infestation that occurs as a result of the feeding of fly larvae in traumatic lesions in the cutaneous tissues of vertebrate hosts [3–5]. It is seen in domestic and wild animals, especially in tropical and subtropical climatic zones. This infestation results in significant economic losses and health issues in farm animals and is also prevalent among humans with low economic and educational development [6]. Species that cause traumatic myiasis are found in the families *Calliphoridae* and *Sarcophagidae* [4,5,7]. In *Calliphoridae*, blowflies consist of moderately large flies that lay eggs into fresh or cooked meat or dung. They are also able to lay eggs on living animals. Wohlfahrtia magnifica, belonging to the *Sarcophagidae* family, is an obligate parasite found in wounds and natural orifices of mammals, including humans. It is common in Europe, Asia, North Africa, the Middle East, and the Mediterranean region [1,8,9].

Although its cases caused by various species have been reported in many animals around the world [4,5,10–12], *Wohlfahrtia magnifica* and *Lucilia sericata* have been reported as the most common species in Türkiye [10,13–16]. In addition, traumatic myiasis cases caused by *Lucilia sericata* and *Calliphora vicina* in wild birds [10,13,14,17–20] and *Chrysomya megacephala*, *Lucilia spp*, and *Calliphora vicina* in turtles have been reported [21–23]. To our knowledge, there have been no reported cases of myiasis in Euphrates soft-shelled turtles and wild goats. To determine the fly species of the larvae,

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certain larval features must be analyzed, which may not always be feasible. In recent years, in these cases, molecular methods have been used to determine the species of larvae [24,25]. MtDNA is a widely used molecular tool in metazoan taxa for taxonomic, population, and evolutionary studies. It has high copy numbers, is easier to isolate, and has a high phylogenetic signal and mutation rates. In recent years, DNA barcoding using CO1 sequences has emerged as an effective tool for molecular identification and phylogenetic characterization of various insect species [26–30].

This study was conducted to evaluate the traumatic myiasis cases detected during the examination of animals brought to Dicle University Wildlife Rescue, Rehabilitation, Training, Practice, and Research Center with wound complaints between 2020-2022. For this purpose, microscopic and molecular identification and characterization of larvae collected from wounds were performed.

2. Material and methods

2.1. Study period and study area

The study was conducted in the Dicle University Wildlife Rescue, Rehabilitation, Training, Practice, and Research Center in Diyarbakır province (37°55′ N, 40°14′ E, 670 m) located in southeastern Anatolia, Turkey between 2020-2022.

2.2. Animals and larvae collection

During the examination of eight wild animals brought with complaints of injury, traumatic myiasis was detected. Before beginning the treatment process, the larvae present in the wound were collected using forceps. These larvae were then stored in 70% ethyl alcohol until species identification and molecular analysis could be carried out. The data concerning the infested animals are illustrated in Table 1. Species identification was carried out as described in the literature [1].

Table 1. Animals' species, date of infections, body location, larvae species in animals suffering from traumatic myiasis.

Animal (no.)	Animal Species	Date	Body Location	Identified Larvae
1	*Buteo buteo*	December 2020	Left Wing	*C. vomitoria, L. sericata*
2	*Buteo buteo*	May 2022	Right Wing	*C. vomitoria, L. sericata, C. vicina*
3	*Ciconia ciconia*	April 2021	Left Wing	*L. sericata*
4	*Buteo buteo*	May 2022	Left Wing	*L. sericata*
5	*Buteo buteo*	April 2022	Left Wing	*L. sericata*
6	*Testudinidae*	June 2022	Under carapace	*L. sericata*
7	*Capra aegagrus*	July 2022	Gluteal	*W. magnifica*
8	*Rafetus euphraticus*	September2022	Under carapace	*L. sericata*

2.3. Molecular analysis

Total genomic DNA (gDNA) isolation from 11 third instar larvae samples with a commercial tissue DNA isolation kit (Thermo Fisher Scientific, Waltham, MA). gDNA samples were stored at -20 °C until use. The region of the cytochrome oxidase subunit I gene (CO1) gene was amplified by PCR using conserved specific primers previously described by Otranto et al. (UEA7 and UEA10) [31]. 9 µl of each PCR product was mixed with 1 µl of TriTrack loading dye (ThermoScientific, R1161) and loaded on 1.4% agarose gel.

COI sequences obtained from larvae were sequenced in both directions on a 3100 ABI PRISM genetic analyzer (Applied Biosystems, Foster City, CA.). Sequences were edited with CodonCode Aligner software version 11.0 (CodonCode Corporation, Dedham, MA, USA). BLAST algorithms and databases from the National Center for Biotechnology (http://www. ncbi.nlm.nih.gov) undertook

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nucleotide sequence analysis. Pairwise calculations and phylogenetic trees were performed with MEGA 7 software [32].

3. Results

The 3rd stage larvae collected from the wounds on the wings of four buzzards (*Buteo buteo*) were identified as *L. sericata, C. vomitria*, and *C. vicina*, and the larvae collected from white stork (*Ciconia ciconia*) were identified as *L. sericata.* It was determined that the larvae obtained from the wild goat (*Capra aegagrus*) were *W. magnifica* larvae, and *L. sericata* larvae were responsible for the traumatic myiasis occurring in the Euphrates soft-shelled turtles (*Rafetus euphraticus*) and turtle (*Testudinidae*) (Table 1 and Figures 1–3).

The eleven sequences obtained have been deposited in GenBank under accession numbers OR642793-OR642793. The average intraspecies nucleotide divergence between *L. sericata* larvae was 0.0018 (0.0000–0.0046) (Table 2). *C. vomitoria* sequences of COI were 100% identical between them. The interspecific divergence between *C. vomitoria* and *C. vicina* was 0.042 (Table 3).

Table 2. Pairwise comparison of nucleotide sequence differences in the COI among *Lucilia sericata* isolates and *Calliphora* spp. isolates in this study.

Species							
Lucilia sericata (OR642795)		0.0024	0.0025	0.0020	0.000	0.0020	0.0020
Lucilia sericata (OR642796)	0.0046		0.0022	0.0015	0.0015	0.0015	0.0015
Lucilia sericata (OR642799)	0.0046	0.0031		0.0016	0.0015	0.0015	0.0016
Lucilia sericata (OR642793)	0.0031	0.0015	0.0016		0.0000	0.0000	0.0000
Lucilia sericata (OR642797)	0.0031	0.0015	0.0015	0.0000		0.0000	0.0000
Lucilia sericata (OR642798)	0.0031	0.0015	0.0015	0.0000	0.0000		0.0000
Lucilia sericata (OR642800)	0.0031	0.0015	0.0015	0.0000	0.0000	0.0000	
Species							
Calliphora vicina (OR642801)				0.0072	0.0073		
Calliphora vomitoria (OR642794)			0.0427		0.0000		
Calliphora vomitoria (OR642802)			0.0431	0.0000			

Figure 1. a- White stork (*Ciconia ciconia*) with wing wound, b- The stork's wing infested with numerous *L. sericata* larvae, c- Ventral view of buzzard wing wound and different species larvae d- The buzzard's wing with fractured and numerous *L. sericata* larvae in wound.

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Figure 2. a- Wild goat (*Capra aegagrus)* with gluteal region wound, b- The wild goats' wound infested with numerous *W. magnifica* larvae, c- Euphrates soft-shelled turtles (*Rafetus euphraticus*) with under carapace wound, e-The Euphrates soft-shelled turtles myiasis caused by *L. sericata*, f- Turtle (*Testudinidae)* with under carapace wound, g- numerous *L. sericata* larvae in wound.

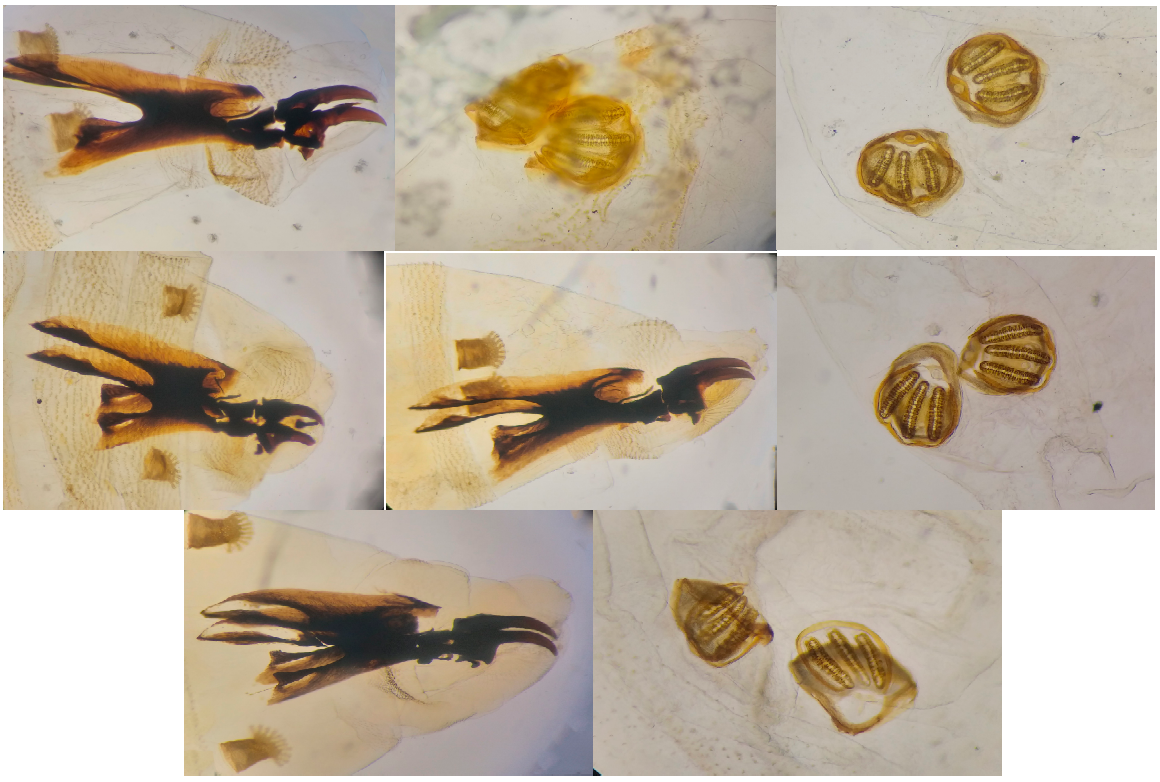

Figure 3. The third stage larvae of *Calliphora vomitoria, Calliphora vicina, Lucilia sericata*, *Wohlfahrtia magnifica* a- *C. vomitoria*, cephalon skeleton, lateral view b- *C. vomitoria*, posterior spiracles c- *C. vicina*, cephalon skeleton, lateral view d- *C. vicina*, posterior spiracles e- *L. sericata*, cephalon skeleton, lateral view f- *L. sericata*, posterior spiracles g- *W magnifica*, cephalon skeleton, lateral view h- *W. magnifica*, posterior spiracles.

4. Discussion

Many injured wild animals, damaged for various reasons, are brought for treatment and recovery to the Wildlife Rescue, Rehabilitation, Training, Application, and Research Centers established in many parts of Turkey, which is the wealthiest country in Europe in terms of wild animal diversity. Traumatic myiasis was detected in 8 animals brought to the Dicle University

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Wildlife Rescue, Rehabilitation, Training, Practice, and Research Center for various reasons such as injury wire entrapment.

Myiasis agents detected in poultry in studies conducted around the world are Calliphora vicina, Lucilia sericata, Lucilia cuprina, Wohlfahrtia vigil, Wohlfahrtia magnifica, Wohlfahrtia opaca, Cuterebra buccata and Dermatobia hominis [18–20,33]. In our country, the species detected in wild birds are L. sericata and C. vicina [10,13,14,17]. In this study, myiasis was detected in two species of wild birds, hawk, and stork, and the species responsible for myiasis were determined to be C. vicina, L. sericata, and C. vomitoria. Cases of traumatic myiasis caused by Calliphoridae reported in hawks worldwide are limited. A case of traumatic myiasis caused by L. cuprina and L. sericata was reported in two Harris' hawks in Peru [34], L. sericata and C. vicina have been reported in hawks in Turkey [10,13,14]. In our study, only L. sericata larvae were found in two of the four hawks, while C. vicina, C. vomitoria, and L. sericata larvae were found in one, and C. vomitoria and L. sericata larvae were found in one. C. vomitoria larvae, which are the causative agent of tertiary myiasis, are rarely encountered in traumatic myiasis cases [5]. Myiasis cases caused by these two Calliphora species in December and April can be explained by the fact that these flies are densely seen in the region during these months [35]. Traumatic myiasis cases caused by L. sericata in white storks have been reported in Austria and Turkey [14,17,36]. In our study, the larvae we collected from the wing wound were determined to be L. sericata.

Euphrates Softshell Turtle *(Rafetus euphraticus) and* Wild Goat *(Capra aegagrus*) have most recently been assessed for *The IUCN Red List of Threatened Species* in 2016 and 2020, respectively. *Rafetus euphraticus* is listed as Endangered under criteria A4c, and *Capra aegagrus* is listed as Near Threatened under criteria A2cd [37,38]

Diagnosis and treatment of any disease in these endangered animals is vital. In this study, Euphrates Softshell Turtle and wild goat, which was examined with complaints of injury, was diagnosed with traumatic myiasis. It was determined that the larvae collected from the Euphrates turtle were *L. sericata*, and the case of traumatic myiasis in the Euphrates turtle was reported for the first time in the world in our study. It was determined that the larvae collected from the wound in the gluteal region of *Capra aegagrus* belonged to *W. magnifica.* To our knowledge, no traumatic myiasis is reported in *Capra aegagrus*, and in this study, traumatic myiasis occurring in *W. magnifica* is presented for the first time.

Molecular analysis of DNA obtained from the larvae identified in this study confirms the morphological diagnosis. As a result of comparing the 644 base pairs obtained from the COI gene of *L. sericata* with the reference sequences, 99.7 to 100% identity was shown with China, Austria, Denmark, UK, and Korea (Figure 4). Intraspecies nucleotide divergence between *Lucilia sericata* larvae in this study was 0.0018 (0.0000–0.0046) and similar to our study, [ao,Fu[27] found the intraspecific divergence of traumatic myiasis caused by *Lucilia caesar* larvae to be 0.0022.

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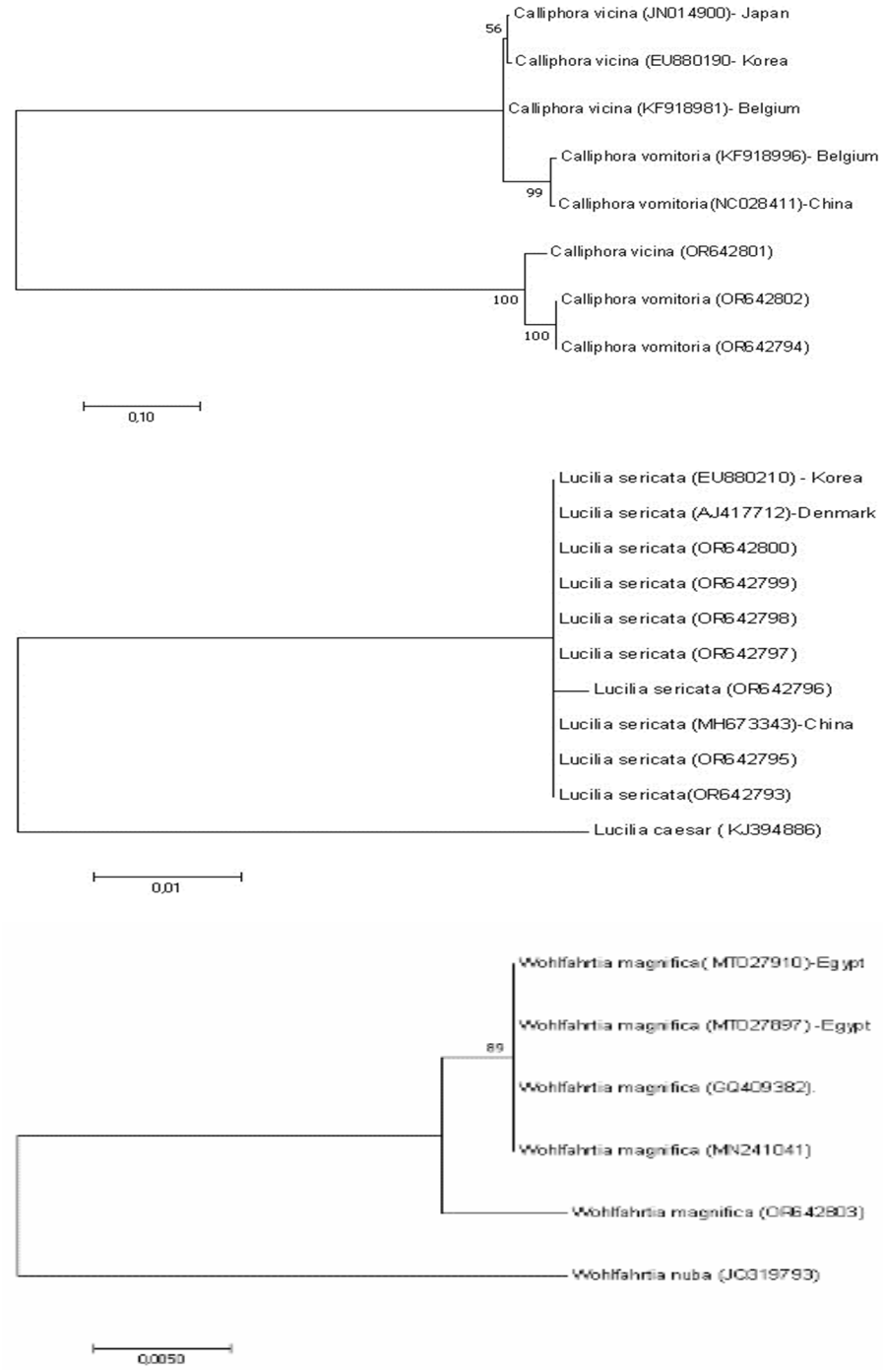

Figure 4. Phylogenetic relationships of *Lucilia*, *Calliphora*, and *Wohlfahrtia* collected from wild animals from Türkiye, inferred by the Maximum Likelihood method of the partial COI gene sequences, based on genetic distances calculated by the Tamura 3-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. All positions with less than 95% site coverage were eliminated, i.e., fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option).

The interspecific divergence between *C. vomitoria* and *C. vicina* was 0.042. Two sequences of *C. vomitoria* were 100% identical between them. The nucleotide sequences were compared with reference sequences from the NIH, and they had an identity of 99.8 to 100%, to *C. vomitoria* from Belgium, China, and the United States and 99.5 to 99.8%, to *C. vicina* from Japan, Belgium, and Korea

(Figure 4). It was determined that the *W. magnifica* larva collected from a single animal showed an identity of 99.52 to 99.44% in Egypt (Figure 4)

5. Conclusion

This study presents myiasis cases caused by different fly species in different wild animals. However, this study reported traumatic myiasis for the first time in a Euphrates turtle. Traumatic myiasis causes serious health problems in many animals, but this infestation, which occurs due to various injuries and can be fatal, especially in endangered wild animals, is significant. In the habitats of wild animals, the number of flies should be reduced by setting traps against flies that cause myiasis, along with precautions to be taken against injuries to these animals.

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Conflicts of Interest: The authors declare no conflict of interest.

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