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First record of traumatic myiasis obtained from forest musk deer (*Moschus berezovskii*)

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ABSTRACT

Myiasis is an infestation of maggots on living tissue in humans and animals all over the world. It is known to occur in wild animals, while no information is reported in forest musk deer (*Moschus berezovskii*). During our research on the conservation of forest musk deer, we found a new record of traumatic myiasis of an injured forest musk deer. The flies are likely *Lucilia caesar* (Linnaeus, 1758) according to the results of DNA barcoding technology. We report traumatic myiasis of forest musk deer for the first time, which expands the information on parasite and myiasis of forest musk deer and confirms the potential risk of traumatic myiasis of forest musk deer.

1. Introduction

Myiasis is the infestation of fly larvae (maggots) in live human or vertebrate animal tissue (Zumpt, 1965; Singh and Singh, 2015). Myiasis-causing flies mainly include Calliphoridae, Muscidae, Oestridae, and Sarcophagidae (Zumpt, 1965; Hall and Wall, 1995; Pezzi et al., 2019), which have caused a major economic problem in animal farming (Zumpt, 1965; Francesconi and Lupi, 2012). However, myiasis of wild animals is an understudied issue, owing to predation or their shelter-seeking behavior (Hall, 1991; Hall et al., 2016). Most methods for diagnosis of myiasis are made by the finding of fly larvae in tissue and identifying fly larvae (Noutsis and Millikan, 1994; Sotiraki et al., 2010). Some alternative identification methods are morphological approaches, molecular approaches, and monoclonal antibody-based enzyme-linked immunosorbent assay (MAb-ELISA) (Azeredo-Espin and Lessinger, 2006; Figarola et al., 2001; Pezzi et al., 2015).

The forest musk deer (*Moschus berezovskii* Flerov) lives in Asia, mainly in China. Although it is currently the most abundant species of musk deer in China, the wild population of forest musk deer is tiny. It has been listed in CITES appendix II and considered as endangered in the IUCN Red List. At present, captivity is the main strategy for the conservation of forest musk deers (Wang and Sheng, 1988; Yang et al., 2003; Meng et al., 2006). On July 6, 2019, we found a case of traumatic myiasis of forest musk deer in Fengchun Musk Deer Breeding Center. Traumatic myiasis is mainly caused by fly larvae developing in animal carrions after female flies directly laying eggs/larvae at the open wounds of humans or animals, resulting in the host wound and surrounding skin appear swelling, inflammation, pain and other health problems (Noutsis and Millikan, 1994; Hall, 1997; Yan et al., 2019). To our knowledge, this is the first report of traumatic myiasis of forest musk deer in China.

Hebert et al. (2003) suggested that DNA barcoding can widely be used in species classification and identification. Therefore, we collected larvae and eggs from the wound and fur of the injured forest musk deer for DNA identification. The aims of our study were (i) to identify the species of larvae and eggs collected from forest musk deer; (ii) to provide more effective information on myiasis of forest musk deer.

2. Material and methods

Fly samples were collected from an open wound on the hindquarter of an adult male forest musk deer in Fengchun Musk Deer Breeding Center (106°54′20.93″E, 34°12′58.07″N; 1496 m), Fengxian County, Shaanxi Province. The wound caused by fighting with other males was surrounded by some fly larvae and eggs (Fig. 1). The larvae and eggs were randomly collected with tweezers and stored in 75% alcohol (McGraw and Turiansky, 2008) at −20 °C for later use. We treated the musk deer with 75% alcohol disinfection and debridement treatment, and the individual later recovered. All larvae and eggs were used to

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Abbreviations

COI	cytochrome oxidase I
PCR	polymerase chain reaction
MAb-ELISA	monoclonal antibody-based enzyme-linked immunosorbent assay
H_d	haplotype diversity

extract DNA respectively with the HotSHOT method (Montero-Pau et al., 2008). DNA was stored at −20 °C after DNA extraction. For PCR amplification, 7 μL of DNA, 1 μL of each cytochrome oxidase I (COI) bidirectional primers (10 μmol/L) and 9 μL of 2 × Es Taq MasterMix (Dye) (Beijing Cowin Bioscience Co., Ltd., China) were used. PCR primers were performed with the universal primers LCO1490 and HCO2198 (Folmer et al., 1994). The PCR procedure was as follows: pre-denatured at 95 °C for 10min, denatured at 95 °C for 1min, annealed at 40 °C for the 30s, extended at 72 °C for 1min 20s, a total of 30 cycles, extended again at 72 °C for 10min, stored at 4 °C. After amplification, 3 μL PCR products were used for 1% Agarose gel (dyed with Goldeview) electrophoresis. The positive product was purified and sent to Beijing Genomics Institute (BGI, China) for bidirectionally sequencing following Zhang et al. (2016).

All sequences were edited and trimmed with BioEdit (version 7.0.9.0) (Hall, 1999), then assembled by SeqMan 7.1.0 (DNAStar, Steve ShearDown, 1998–2001 version, DNASTAR Inc., USA). The obtained sequences were aligned with sequences available in the GenBank database using the BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). We used MEGA 7 (Kumar et al., 2016) to align our fly sequences together with known sequences of *Lucilia* and *Calliphora* in NCBI, to compare interspecies and intraspecies nucleotide divergence with Kimura's two parameter model (Kimura, 1980), and to construct neighbor-joining (NJ) tree with *p*-distance model (Saitou and Nei, 1987; Nei and Kumar, 2000). All the COI-related of *Lucilia* and *Calliphora* were downloaded from NCBI. We used iTOL (Letunic and Bork, 2019) to visualize the NJ tree.

3. Results

A total of 49 sequences were obtained for five larvae and 44 eggs (Genbank accession no. **MZ165284**–**MZ165332**).

The nucleotide divergence between the eggs/larvae and *Lucilia illustris* (Meigen) (Diptera: Calliphoridae) was 0.0143, and the nucleotide divergence between the eggs/larvae and *Lucilia caesar* (Linnaeus) (Diptera: Calliphoridae) was 0.0054 (Table S1). The interspecific variation rates ranged from 1.43% (*L. caesar* and *L. illustris*) to 11.25% [*Lucilia purpurascens* (Walker) (Diptera: Calliphoridae) and *Lucilia papuensis* Macquart (Diptera: Calliphoridae)]. The average intraspecies nucleotide divergence between eggs and larvae was 0.0022 (0.0000–0.0164) (Table S2).

The NJ tree suggested that different species can be distinguished. Our sample clustered together with *L. caesar* and were nested away from the *L. illustris* (Fig. 2). The value of bootstrap was 93. Only two *L. illustris* nested in *L. caesar* and six *L. caesar* nested in *L. illustris*, and these sequences are not from our fly samples in this study. The detailed information of the whole NJ tree was in Additional file 2.

4. Discussion

Analyses showed that the eggs and larvae collected from the wounds of musk deer are the same species (nucleotide divergence is 0.0022). Hebert et al. (2003; 2004) proposed that the interspecific nucleotide divergence should exceed 3%. Boehme et al. (2012) suggested that the intraspecies difference of *L. caesar* was lower than 1.17%, and the interspecies difference of *L. caesar* and *L. illustris* was 1.17%–1.96%. The nucleotide divergence between our samples and *L. illustris* is 0.0143, and between our samples and *L. caesar* is 0.0054. NJ tree showed that sequences obtained in this study all clustered with *L. caesar*. *L. caesar* and *L. illustris* could not be identified completely accurately because they formed a polyphyletic group. Taken together, the blowfly eggs and larvae collected from the wound are very likely *L. caesar*.

Diagnosis and prevention of traumatic myiasis are extremely important because it seriously threatens the host health and causes significant economic losses to the livestock industry, and the main control strategies for myiasis are light trapping and conventional chemical control (Sotiraki et al., 2010; Yan et al., 2019). Therefore, for the long-term health of captive wild animals, we could use physical methods to prevent and control traumatic myiasis according to the living habits of blowflies, such as fly traps with carrion baits, sticky traps and light trapping. Besides, we also should monitor the health status of the animals themselves, promptly treat and nurse wounds, avoid wound worsening. In the 20th century, the musk deer (*Moschus moschiferus*) in the Sikhote-Alin Mountains found serious cutaneous myiasis caused by *Booponus inexpectatus* Grunin (Diptera: Calliphoridae) forming subcutaneous warbles (Rognes, 1998). Here, we indicated that flies of Calliphoridae can cause severe myiasis in musk deers, and the status of myiasis in forest musk deer is neglected.

5. Conclusion

This study reports the first case of traumatic myiasis in forest musk deer. This traumatic myiasis is not an accidental event, which indicated the potential risk of forest musk deer of blowfly infection for captive and wild individuals. Traumatic myiasis can cause some problems of health, but it is neglected in forest musk deer. We should attach importance to the myiasis of forest musk deer, and we can construct some traps with carrion baits and sticky traps in Breeding Center to trap blowflies, reducing the number of pest flies.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

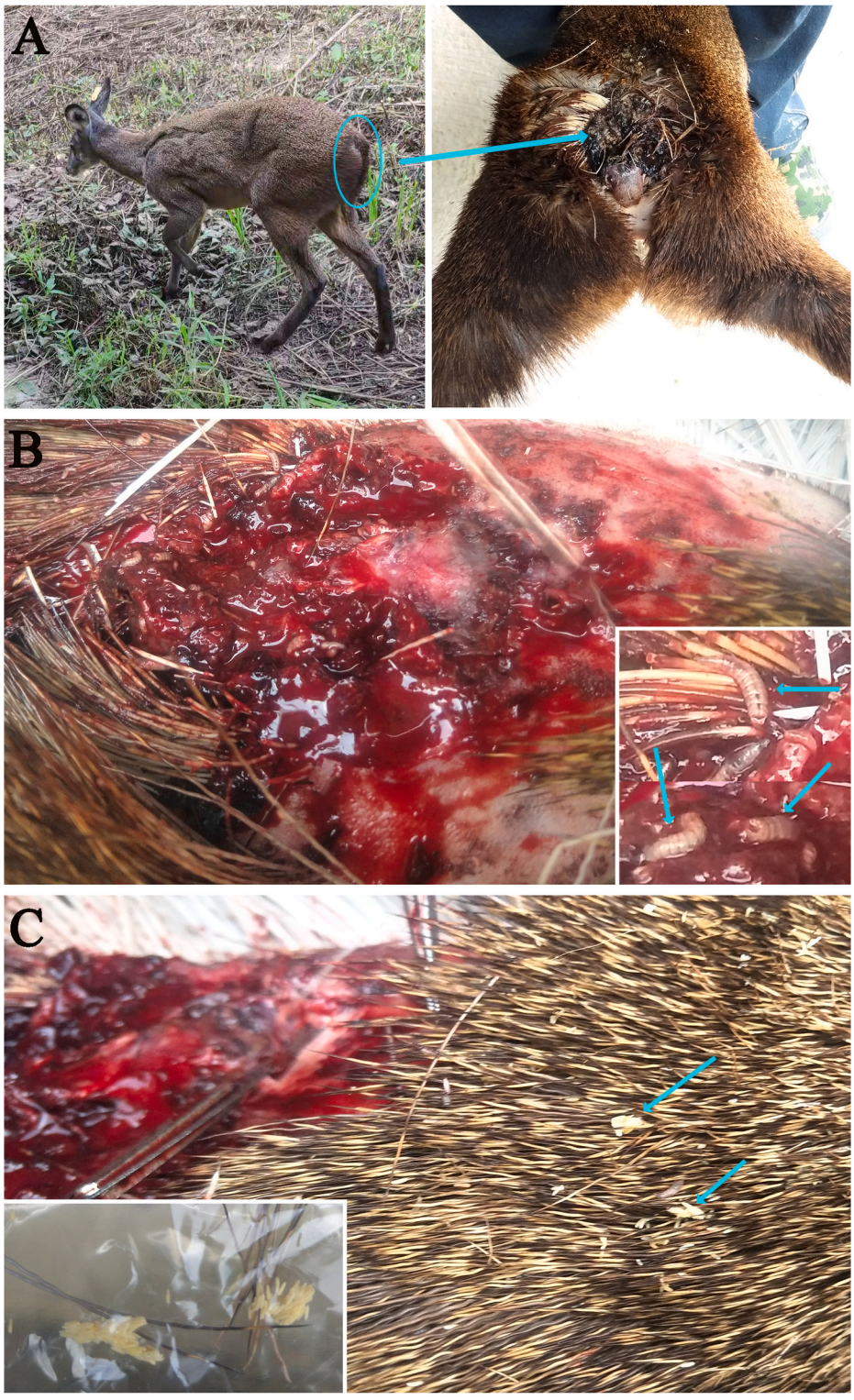

Fig. 1. The myiasis of an open wound of a male Forest Musk Deer. The Forest Musk Deer is breeding in Shaanxi Fengxian Fengchun Musk Deer Breeding Center. The wound is oval, located on the hindquarter. A. Wound site of forest musk deer. B. Blowfly larvae were found in the wound after the wound was cleaned. C. Egg clusters on the fur of forest musk deer.

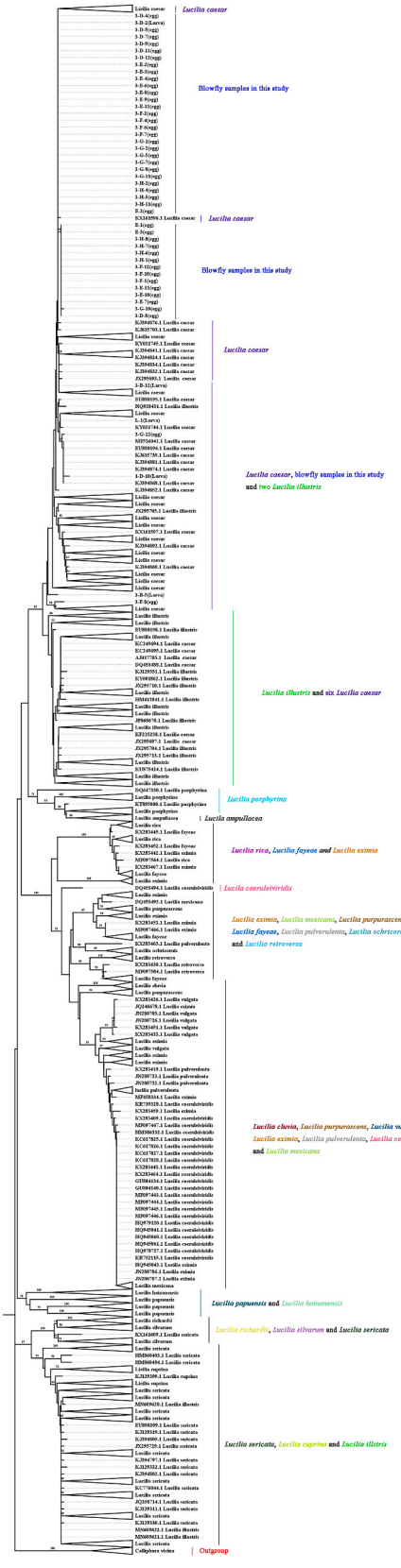

Fig. 2. The neighbor-joining (NJ) tree (500 bootstrap replicates) generated using MEGA 7 with *Lucilia* DNA barcoding fragments based on *p*-distance Model. Our blowfly samples, *L. caesar* and *L. illustris* formed a polyphyletic group. Bootstrap support values below 50 are hidden at the nodes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.08.004.

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