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Original Research Article

Accidental myiasis caused by housefly (*Musca domestica*) in Rock Hyrax (*Procavia capensis Jayakari*) in Saudi Arabia

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Abstract

Purpose: *To determine the organisms responsible for intestinal infection in rock hyrax (Procavia capensis jayakari), a wild animal in Saudi Arabia, through morphological and molecular characterization.*
Methods: *Intestinal contents obtained from 20 dead rock hyrax were used to confirm the presence of larvae. Larval morphology was examined using stereo-microscopy and scanning electron microscopy (SEM). For molecular characterization, ribosomal 18S rDNA and mitochondrial DNA (mtDNA) gene sequences in larval DNA were amplified by polymerase chain reaction (PCR).*
Results: *Four samples of the intestinal contents of dead rock hyrax showed positive response indicative of larval infection (infection rate 20 %) with a slender and creamy larval structure in rock hyrax intestine. For molecular characterization, from the 20 samples, 11 ribosomal 18S rDNA sequences and six mitochondrial DNA (mtDNA) sequences presented positive polymerase chain reaction (PCR) signals. The gene sequences were used to construct an evolutionary tree with other related sequences available in a gene database. The morphological features and the evolutionary trees were developed based on the above two genes, confirming the resemblance between Musca domestica (Housefly) and larvae in this study.*
Conclusions: *The intestine of rock hyrax is invaded by houseflies. The results obtained could be a first step in understanding the cause of myiasis infection in wild animals. The translational value of these findings raises a serious concern regarding the transmission of this disease in animals with houseflies being the vector for pathogens to enter hosts such as wild animals. This would pose a serious problem in preserving the ecological balance of wild animals in the future.*

Keywords: *Musca domestica, Procavia capensis jayakari, Accidental myiasis, Scanning electron microscope, Polymerase chain reaction*

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INTRODUCTION

Myiasis is a parasitic infection of dipterous larvae and has been reported in humans, animals and poultry but rarely in amphibians and reptiles. The larval stages of dipterous fly, commonly known as maggot or grub, infect the organs or tissues of host animals. The larvae feed on necrotic or live tissue, liquid body material, or food directly from the host. Depending on the body condition and interaction between larvae and host, it causes a wide range of intestinal infections [1]. Recently, Salem and Attia reported “accidental myiasis” in broiler chicks in Egypt characterized by diarrhea symptoms caused by the larvae [2]. In animal husbandry, these species are largely of medical

concern and responsible for major losses, such as low milk production, weight loss, reproductive difficulties and poor quality, which results in significant economic losses [1].

Musca domestica, commonly known as housefly, lives near humans and animals and is found in hospitals, restaurants, food waste centers and food storage areas. Housefly causes serious epidemiologic disruption such as acute gastroenteritis, especially in young children in developing nations, because they carry helminthic eggs, *Ascaris lumbricoides* and *Trichuris trichiura* [3]. This fly causes irritation and gastrointestinal and traumatic myiasis in humans and animals [4]. Myiasis host-parasite connections are likely to be of great concern because of their serious impact on poultry and animal husbandry. The causal agent belongs to the fly family that causes myiasis and its potential pathogenic consequence known as "accidental myiasis" has led to ecological disturbances in poultry and animal husbandry [5]. Furthermore, myiases are often categorized based on the larval invasion of the host tissue and their location. They are classified as dermal, subdermal (*Hypoderma bovis* and *Hypoderma lineatum*), gastric (*Gastrophilus* spp.), cutaneous, nasopharyngeal, or ocular (*Oestrus ovis*). In addition, they have also been reported in the intestinal or urinogenital tract (*Hypoderma* spp) [6]. Studies indicate that the error in diagnosing the type of myiasis may lead to taking unnecessary measures because it is sometimes considered an accidental infection in wild animals and symptoms are similar to that of other injuries, leading to wrong treatment. Several nucleic acid-based techniques have been developed for the identification and characterization of diverse species. Molecular indicators, such as cytochrome oxidase I (COI), cytochrome oxidase subunits II (COII) and ribosomal DNA genes, have been used for molecular characterization of species and genera. In vertebrates, insects and other taxa, COI contains highly conserved sequences and various sections [7]. This study was conducted using small wild mammals (*Procavia capensis jayakari*) to describe myiasis resulting from *Musca domestica* larvae recovered from the intestinal tract of dead animals. Molecular and morphological characterization of the larvae causing myiasis in the rock hyrax (*P. capensis jayakari*) were performed.

METHOD

Sample collection

The intestinal contents of 20 rock hyraxes (*P. capensis jayakari*) were collected from animals that died recently due to various causes between the period April 2021 to June 2022. The dead animals were obtained from open mountainous areas in Howtat Bani Tamim province (23.50474, 46.85241), close to the natural reserve (Ibex Reserve) south of Riyadh, Kingdom of Saudi Arabia (Figure 1).

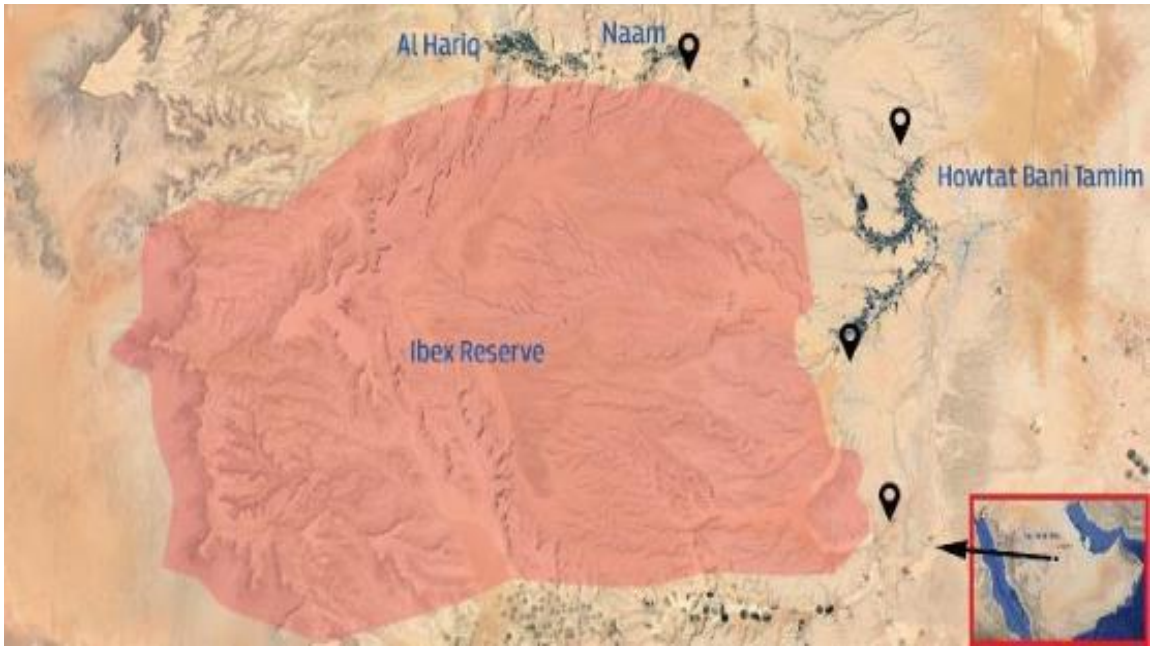

Figure 1: The sites for collecting samples from the intestines of rock hyrax and it is noted that study area is close to the natural reserve (Ibex Reserve) https://www.google.com/maps/@23.3999478,46.6842707,53456m/data=!3m1!1e3?entry=ttu

Morphological characterization of larvae

Fly larvae and the intestinal contents of the dead animals were examined immediately after autopsy. The larvae were preserved in 70 % ethanol and stored in a refrigerator for morphological study. Subsequently, they were first examined under a stereo-microscope (OMAX, China) at 10x magnification and the dimensions of larvae were recorded [8]. For morphological examination using the scanning electron microscope (SEM), the larvae were collected and stored at 4 °C . Thereafter, they were rinsed multiple times with phosphate buffer (pH 7.2), dried on Whatman No. 1 filter paper (Merck Life Science UK Limited, Dorset, UK) and examined by SEM according to Mahdy and Attia's methodology [9]. The samples were coated with 20 nm gold in a sputter coater (Q150R S sputter-coated, England) and SEM was carried out in a high-resolution microscope (FEI Quanta FEG 250, FEI Company, Czech Republic) at the Central Instrumentation Facility, College of Science and Humanities, Prince Sattam bin Abdulaziz University, Saudi Arabia.

DNA extraction and PCR amplification

Extraction of DNA from the stored larvae (~ 50 mg) was performed using the DNA Mini Kit blood/tissue kit (MEBEP Bioscience, China), following the manufacturer's instructions. Amplification was set up using 2x PCR super master mix buffer containing dNTPs, 2 mM $MgCl_2$, DNA Taq polymerase, DNA template (3.0 µL) and primers (with a volume of 12.5 µL and 1.0 µL, respectively, for forward and reverse primers). The reaction master mixture was adjusted to a final volume of 25 µL with DNase-free water. Primers for 18S rDNA and COI genes, listed in Table 1, were obtained from previous studies.

Furthermore, PCR amplifications were carried out in a thermal cycler (Eppendorf Mastercycler, Hamburg, Germany) with the following parameters: initial denaturation (4 min at 95 °C), denaturation (94 °C for 30 s), annealing (30 s at 58.5 °C for 18S rDNA; 60 s at 50 °C for COI), and an extension (72 °C for 60 s), with a final extension (72 °C for 5 min) for 35 cycles. The amplicons were stored at 4 °C and further subjected to gel electrophoresis for visualization. The electrophorized amplicons were observed using a gel documentation system (Gel Doc XR+ System, Bio-Rad, Singapore). The sequencing of 18S rDNA and COI from the PCR products was performed using an automated sequencer (Applied Biosystems ABI 3730xl 96-capillary DNA analyzer) from the Macrogen sequencing facility (Macrogen Inc., Seoul, South Korea).

Data analysis

The sequence data of 18S rDNA and COI were analyzed using the NCBI BLAST program and the open resource database (BLAST: Basic Local Alignment Search Tool (nih.gov)) was used to confirm the sequences. Based on the BLAST results, a phylogenetic tree was constructed to determine genetic diversity and similarity between the sequences against the nucleotide database.

The nucleotide sequences of 18S rDNA obtained were deposited in GenBank under the accession numbers (OP684320, OP684321, OP684322, OP684323, OP684324, OP684325, OP684326, OP684327, OP684328, OP684329 and OP684330) and compared with three strains of *M. domestica* (KC177313.1, GQ465780.1 and AF322423.1) and other taxa belonging to different groups viz *Lucilia sericata* (KR133393.1 and XR 005232261.1), *Lucilia sp.* VH-2001 (AF322425.1), *Calliphora nigribarbis* (AB466039.1), *Atylostoma towadensis* (AB466022.1), *Panzeria melanopyga* (AB466021.1), *Suensonomyia nudinerva* (AB466016.1), *Melinda viridicyanea* (AF322424.1), *Ectophasia rotundiventris* (AB466034.1), *Parasetigena silvestris* (AB466001.1), and *Frontina laeta* (AB466008.1). Similarly, the nucleotide COI sequences were deposited in GenBank under the accession numbers (OP684335, OP684336, OP684337, OP684338, OP684339, and OP684340).

Table 1: List of primers used for amplification of 18S rDNA and COI genes

Gene	Primer	Direction	Sequence	Reference
18S rDNA	18SA	Forward	AACCTGGTTGATCCTGCCAGT	[10]
	18SB	Reverse	TGATCCTTCCGCAGGTTCACCT	
COI	TY-J-1460	Forward	TACAATTTATCGCCTAAACTTCAGCC	[11]
	C1-N-2800	Reverse	CATTTCAAGCTGTGTAAGCATC	
	C1-J-1751	Forward	GGATCACCTGATATAGCATTCCC	[12]
	TL2-N-3014	Reverse	TCCAATGCACTAATCTGCCATATTA	

They were also compared with the other strains of *M. domestica* (JX861432.1, AY526196.1 AND KT272839.1) and other taxa belonging to different groups including *Chrysomya chani* (FJ195377.1), *Chrysomya marginalis* (KM434352.1), *Chrysomya greenbergi* (FJ195385.1), *Chrysomya bezziana* (MK167359.1), *Haematobia irritans exigua* (AB479526.1), *Hermetia illucens* (GQ465783.1) and *Fannia canicularis* (KC249710.1) for the construction of a phylogenetic tree.

The evolutionary relationship between the nucleotide sequences obtained from this study and those of other genera was analyzed using the Maximum Likelihood and Neighbor-Joining methods following the Tamura-Nei model [13,14]. The replicates were used under bootstrap consensus for phylogenetic analyses. In this study, less than 50 % of bootstrap replicates were collapsed branches corresponding to partitions and replicate tree-associated taxa clustered with bootstrap test (1000 replicates) present next to the branches. The Maximum Composite Likelihood technique was used to calculate evolutionary distances. They were displayed in proportion to the number of base substitutions per site, with ambiguous regions excluded for each sequence pairing (pairwise deletion option).

In this experiment, 11 vs 16 (from the dataset used as a control) nucleotides for 18S rDNA and 6 vs 10 (from the dataset used as a control) COI were used for comparative study between the group using MEGA11 program [15].

RESULTS

Morphological description

Four samples of the intestinal contents of dead rock hyrax from the total of 20 samples collected contained larvae (20 % infection rate). The larvae were slender, creamy white, 10 - 15 mm in length (average of 13.3 ± 0.38 mm), showing typical Diptera features. The cephalic region (cr), which is visible on the larval body, had a straightforward pair of sclerotized mouth hooks. SEM result showed that the larvae had eight abdominal segments (aI-aVIII), a last segment with two spiracles, three thoracic segments (tI-tIII) and the first thoracic segment with six spiracles at its anterior end (Figure 2 A).

The descriptive view of SEM study was further examined to validate the involvement of the *M. domestic* larva in myiasis. The larvae have a completely formed cephalic area according to the SEM results (Figure 2 A). The top of the cephalic region has antennae, maxillary palps, oral ridges, cirri and an oral opening. The cephalic collar spines are unique, establishing a uniform sequence in the laterally and ventral segments, while the rows are asymmetrical and constitute a clump of squashed spines in the dorsal area (Figures 2 B and C).

Anterior spiracles, a regular row of six ramifications on either side of the first thoracic segment, are present (Figures 2 D and E). Transverse crevice, lateral creeping welts, and ventral creeping welts with spines are all visible on the abdominal segment (Figure 2 F) while the anal aperture and papillae are highlighted by the anal pad (Figure 2 G). The two spiracular plates in the eighth segment are seen in the posterior area of the larvae (Figure 2 H). Finally, three spiracular apertures, spiracular scars and peristigmatic tufts are observed on the posterior spiracle (Figure 2 I).

Molecular characterization and Phylogenetic analyses

Genomic DNA was isolated from eleven larval samples and PCR amplification of the 18S rDNA was done. The length of the PCR product and sequences from the 11 samples were 1742 bp. For the cytochrome oxidase I (COI) gene, DNA was amplified from six samples and the amplified gene sequence was 1497 bp. Maximum-likelihood (ML) and neighbor-joining (NJ) phylogenetic analyses of the 18S rDNA sequencing data from myiasis larvae in this study revealed that they belonged to Diptera and were associated with three strains of *M. domestica* (KC177313.1, GQ465780.1 and AF322423.1). Other taxa or a few different groups were *Lucilia sericata* (KR133393.1 and XR 005232261.1), *Lucilia* sp. VH-2001 (AF322425.1), *Calliphora nigribarbis* (AB466039.1), *Atylostoma towadensis* (AB466022.1), *Panzeria melanopyga* (AB466021.1), *Suensonomyia nudinerva* (AB466016.1), *Melinda viridicyanea* (AF322424.1), *Ectophasia rotundiventris* (AB466034.1), *Parasetigena silvestris* (AB466001.1) and *Frontina laeta* (AB466008.1) from GenBank (Figure 3).

This analysis revealed that all the sequences obtained from the samples in the present study were 99.88 %, 99.88 % and 99.59 % identical with the three strains: KC177313.1, GQ465780.1 and AF322423.1 of *M. domestica*, respectively. For the other groups, the similarity ranged between 97.98 % and 97.17 % (Table 2).

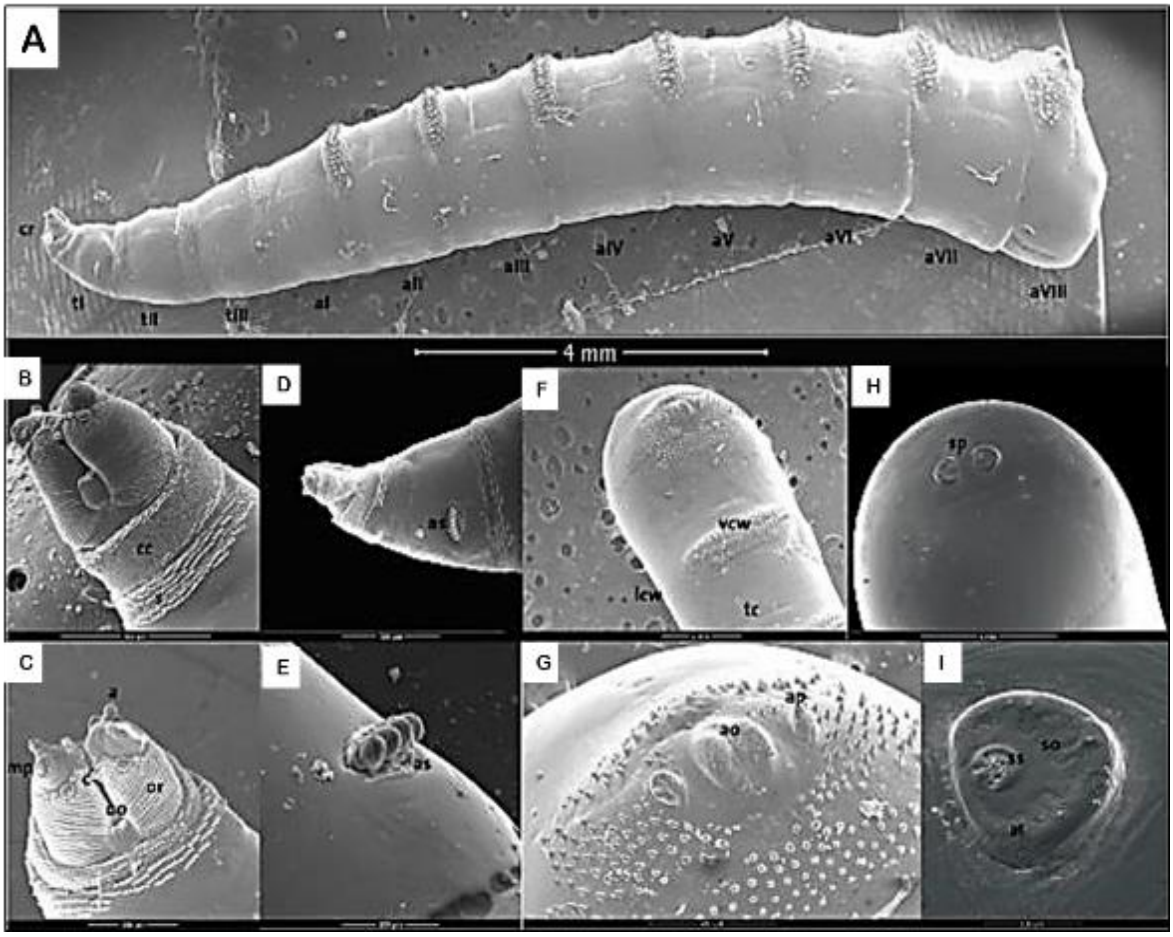

Figure 2: Larvae of *Musca domestica* (Diptera: *Muscidae*): (A) Larval body; from left, cephalic region (cr), three thoracic segments (tI–tIII), and eight abdominal segments (aI–aVIII). (B) Details of the spines of the cephalic collar (cc) and the spines (s) of the anterior part of the first thoracic segment (tI). (C) Detail of the cephalic region with the antennae (a), maxillary palps (mp), oral ridges (or), cirri (c) and oral opening (oo). (D & E) First thoracic segment highlights the anterior spiracle (as), one on each side. The anterior spiracles (as) have a regular row with six ramifications. (F) Ventral view of the fifth and sixth abdominal segment, showing the ventral creeping welts (vcw) with spines all over the surface, the lateral creeping welts (lcw) and the transverse crevice (tc). (G) Detail of the anal pad highlighting the anal opening (ao) and the anal papillae (ap). (H) Posterior region (pr) of the larvae shows the two spiracular plates (sp) at the eighth abdominal segment. (I) Detail of the posterior spiracle showing three spiracular openings (so), spiracular scars (ss) and peristigmatic tufts (pt)

The 11 nucleotide sequences of 18S rDNA obtained from this study were deposited in NCBI GenBank database under the following accession numbers (OP684320, OP684321, OP684322, OP684323, OP684324, OP684325, OP684326, OP684327, OP684328, OP684329 and OP684330).

In addition, Maximum-likelihood (ML) and neighbor-joining (NJ) phylogenetic analyses of the COI locus sequencing data from myiasis-causing larvae revealed that they belonged to Diptera and were associated with three strains of housefly (JX861432.1, AY526196.1 and KT272839.1). Other taxa or a few different groups included *Chrysomya chani* (FJ195377.1), *Chrysomya marginalis* (KM434352.1), *Chrysomya greenbergi* (FJ195385.1), *Chrysomya bezziana* (MK167359.1), *Haematobia irritans exigua* (AB479526.1), *Hermetia illucens* (GQ465783.1) and *Fannia canicularis* (KC249710.1) from GenBank have been shown in Figure 4.

The results of this analysis showed that every sequence generated from the materials used in this investigation was ~100 % similar to that of *M. domestica* (JX861432.1, AY526196.1 and KT272839.1; Table 3). The similarity for other groups varied between 97.10 % and 90.18 %. The six nucleotide sequences of COI obtained from six different samples in this study were deposited in NCBI GenBank database with the corresponding accession numbers (OP684335, OP684336, OP684337, OP684338, OP684339 and OP684340).

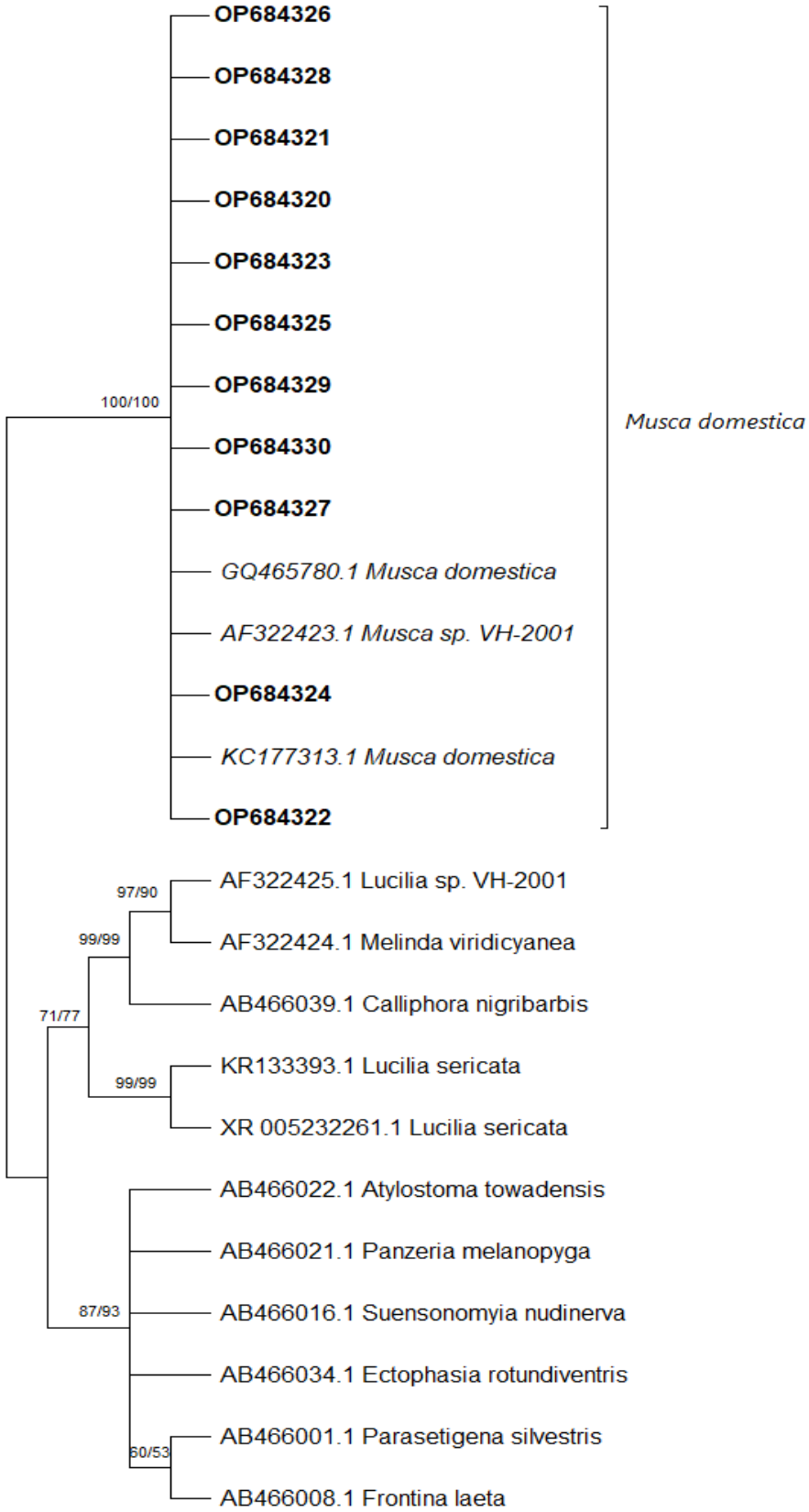

Figure 3: Comparative phylogenetic relationships tree among the obtained 11 larvae sequence in this study (Bold) and some other Diptera genera (14 nucleotide sequence) based on 18S rDNA by using the Maximum Likelihood method and Neighbor-Joining method (ML/NJ) through MEGA 11

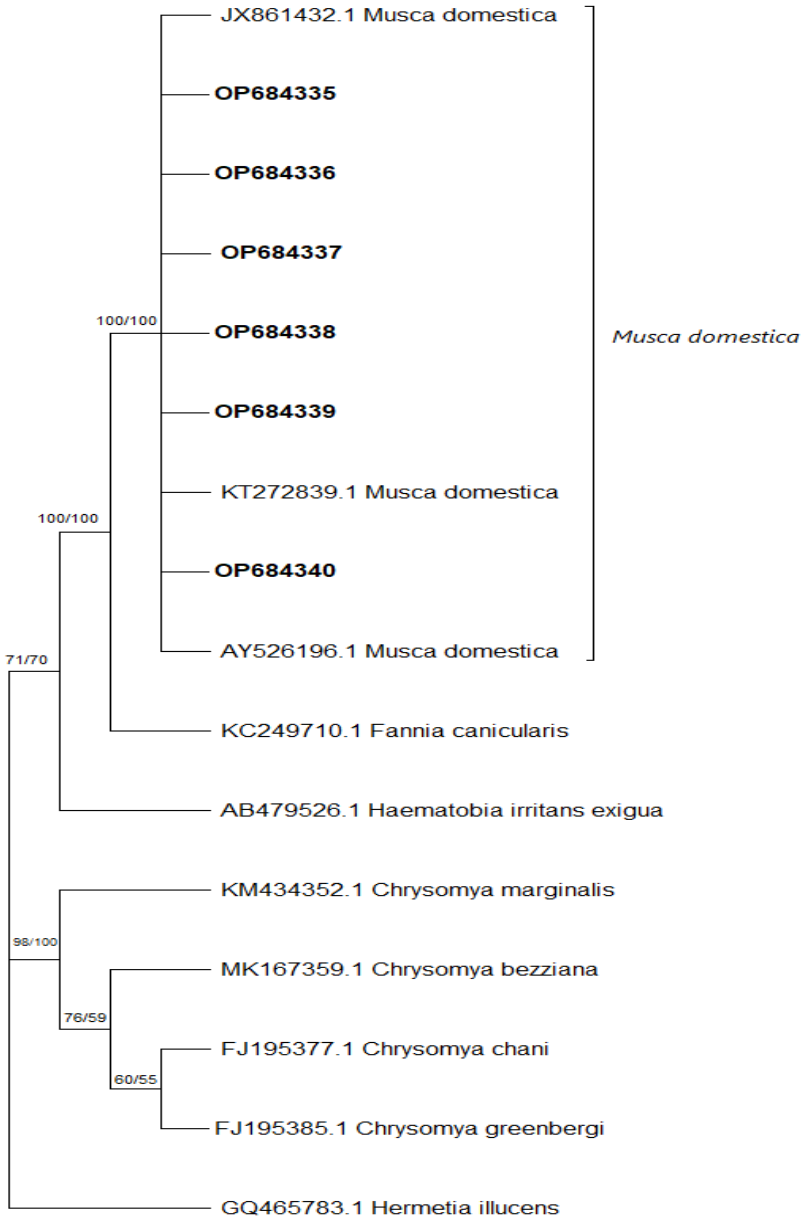

Figure 4: Comparative phylogenetic relationships tree among the obtained 6 larvae sequence in this study (Bold) and some other Diptera genera based (10 nucleotide sequence) on COI by using the Maximum Likelihood method and Neighbor-Joining method (ML/NJ) through MEGA 11

Table 2: Similarity percentage for 18S rDNA between the identified larvae in rock hyrax and other strains of *Musca domestica* from database

Seq ID accession number	Accession number of Musca domestica Strain		
	KC177313.1	GQ465780.1	AF322423.1
OP684320	99.88	99.88	99.59
OP684321	99.77	99.77	99.48
OP684322	99.94	99.94	99.65
OP684323	99.77	99.77	99.48
OP684324	99.88	99.88	99.59
OP684325	99.71	99.71	99.42
OP684326	98.15	98.15	97.86
OP684327	99.59	99.59	99.31
OP684328	99.42	99.42	99.13
OP684329	99.83	99.83	99.54
OP684330	99.54	99.54	99.25

Table 3: Similarity percentage for COI between identified larvae in rock hyrax and other strains of *Musca domestica* from database

Seq ID accession number	Accession number of *Musca domestica* Strain		
	KT272839.1	AY526196.1	JX861432.1
OP684335	100	100	100
OP684336	100	100	100
OP684337	100	100	100
OP684338	100	100	100
OP684339	100	100	100
OP684340	99.73	99.73	99.73

DISCUSSION

In recent past, analysis using the electron microscope, specifically Scanning Electron Microscopy, has been useful for investigating external features of parasites in many hosts. In this study, morphological features of larvae of *M. domestica* were studied by SEM and description obtained are in consonance with previous studies that defined the larval stages in *M. domestica*. These studies presented the descriptions of all instars of the housefly [16].

In addition to causing dermal myiasis in sheep, records from the Middle East show that majority of human myiasis cases, known as facultative myiasis, are caused by *M. domestica*. Accidental myiasis, which is caused by *M. domestica,* has been reported in both animals and humans [1,17]. In the past, two individuals with intestinal myiasis were studied in addition to another human record [18]. Moreover, accidental intestinal myiasis has been reported in children and it is caused by contamination of food and water by domesticated houseflies [1,19]. In addition, temperature and humidity play an important role in creating an environment that favors the growth and breeding of *M. domestica* adults in broiler chicken farms. As infected ingested material passes through the digestive tract, eggs hatch and larvae are discharged into the alimentary tract and poor management system may be contributory [2].

Based on some previous reports, which implicated *M. domestica* as a possible cause of myasis, it is plausible that the larvae recovered in the present study could have some pathological effect on the intestinal tract of the rock hyrax. The mechanism by which *M. domestica* larvae are transmitted to the rock hyrax could be due to its wild nature, however, it may be transmitted though the waste food and urbanization of the forest [20,21].

The morphological methods have some limits for characterization of the organisms, therefore, a second line of evidence using PCR amplification of larva DNA was used for further confirmation of the larvae in the rock hyrax. The diagnosis was made via the PCR identification of ribosomal 18S rDNA and mitochondrial DNA genes. Results show that myiasis of rock hyrax was caused by larval infection by *M. domestica*. In recent years, molecular characterization and phylogenetic studies of insect species have mostly been conducted using PCR, which primarily targets nuclear and mitochondrial genes. To ascertain the connections within the subfamily Psychodinae in various parts of the world, cytochrome b, NADH dehydrogenase subunit 1, ribosomal RNA genes (28S, 18S and 16S), mt-COI and mt-COII have been employed for the identification of correct accessions [22]. Molecular investigations of mtDNA have provided diagnostic markers for species identification in several insect categories.

The mitochondria-encoded genes, COI and COII, are among the most reliable and strong molecular markers for identification and phylogenetic analysis in majority of insect species, because this gene area includes highly conserved sequences and varied sections in vertebrates [7,23]. Similar study has also been conducted on *Hypoderma* species. Also, PCR-RFLP (PCR-restriction fragment length polymorphism) analysis targeting COI of mtDNA is used for molecular identification of the parasite and studying differentiation of the most common *Hypoderma* species [24]. Based on the sequences and phylogenetic relationship with 18S rDNA and COI genes, the larvae were confirmed as *M. domestica* larvae. The results for 18S rDNA (99.88 %) and COI (100 %) have similarities with the stains of *M. domestica* (Table 3) which have been published in NCBI database by several investigators [25].

Interestingly, this is the first report of intestinal myiasis implicating *M. domestica* larvae in rock hyrax (*P. capensis Jayakari*), an herbivorous animal that feeds on a wide variety of plants, including leaves, stems, fruits and buds. Hyraxes also climb trees and feed on their leaves. Grasses comprise approximately 75 % of the diet of rock hyrax, including *Acacia tortilis* and *Acacia ehrenbergiana*. Rock hyrax inhabits steep rocky

areas and urinates and defecates in specific places, producing dumps of feces and dried urine [26]. Irrespective of the animal's behavior and habits, it could be infected with myiasis from houseflies which were not existent in its habitat, most likely as a result of the convergence of its environment especially with the human environment. Furthermore, a lack of rural waste management has a negative impact on animals in regions surrounding communities [27-29].

CONCLUSION

This study is the first report to demonstrate the ability of houseflies to invade the intestine of rocky hyrax in Saudi Arabia. Natural biodiversity loss has occurred in millions of hectares during urban expansion. Identical socioeconomic route scenarios would induce disproportionately significant natural habitat dispersion in future. Furthermore, rural garbage has exerted a negative impact on animal species and their habitats, this is because of a significant disruption in animal onshore and aquatic ecosystems. The main cause of rural waste pollution in wildlife is lack of waste management systems in rural areas, enhancing the growth of pathogens. The results of this study pave way for conducting further studies to understand the cause of this infection in wild animals and to identify human involvement. Future investigation needs to be performed to reveal possible transmission of this pathogen, its mode of action and its effect on ecological balance.

DECLARATIONS

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Ethical approval

The Ethical Committee of the Prince Sattam bin Abdulaziz University approved all experiments involving dead animals in accordance with the relevant guidelines, regulations, legal requirements and ethical standards.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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