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Molecular detection and genetic diversity of *Otodectes cynotis* in Domestic Cats in Mosul, Iraq

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Abstract

Background: *Otodectes cynotis* is a common ectoparasite in domestic cats and represents an important cause of feline otoacariasis. Molecular characterization may contribute with additional evidence to the parasite identification as well as its genetic comparison.

Purpose: The current work was carried out to investigate *O. cynotis* in Mosul city on domestic cats by using conventional and molecular methods.

Methods: The present study was carried out on 85 domestic female cats for infestation of *Otodectes cynotis* in veterinary clinics in Mosul, Iraq, during the period from the 1st of October, 2025, to the 30th of April, 2026. DNA was extracted from microscopically confirmed infested cat samples and amplified by PCR with the LCO1490 and HC02198 primers targeting a partial fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Some PCR products were sequenced and phylogenetically analyzed.

Results: *Otodectes cynotis* was detected microscopically in 12 of the 85 examined cats (14.12%; 95% confidence interval [CI]: 8.26%–23.07%). All 12 microscopy-positive samples were subsequently confirmed by PCR, representing a molecular confirmation rate of 100% (12/12) among microscopy-positive cats. Three PCR-positive samples were selected for sequencing and deposited in GenBank (accession numbers PZ715811, PZ715812, and PZ715813). BLASTn analysis revealed 99.39–100% nucleotide identity with reference sequences of *O. cynotis*.

Conclusion: *Otodectes cynotis* was detected in cats from Mosul, Iraq, and three COI sequences were obtained for confirmation of molecular identification. However, three isolates are not enough to describe the genetic diversity in the population. Future studies should include cats of both sexes, different ages, and from different localities.

Keywords: COI gene, domestic cats, genetic diversity, *Otodectes cynotis*, PCR, phylogenetic analysis.

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Introduction

Otodectes cynotis (Acari: Psoroptidae) is an ectoparasite that parasitizes on domestic cats and is one of the agents causing infestation of cat ear mites. An ear mite infestation may cause scratching, shaking of the head, redness, and accumulation of ear wax in the external ear canal. The ectoparasite has been reported in numerous locations and environments in connection with the cat population (Fanelli *et al.*, 2020; El-Dakhly *et al.*, 2025; Deniz *et al.*, 2026). Although wild cats were considered the sole hosts of the mite, it was detected in the ear of a domestic cat as well (Briceño *et al.*, 2020).

The first examination of the external ear canal followed by a microscopic identification of the mite in ear material is usually employed for the clinical diagnosis of *O. cynotis*. On examination, the parasite can be recognized by the characteristic set of morphological features that are typical of the classic microscopic method. However, it should be remembered that the amount and quality of the collected material and the technique used also have a significant impact on the diagnostic accuracy. Likewise, the otoscopic and aural findings are of clinical interest but less effective than the above-mentioned diagnostic methods. The comparative study of the techniques to diagnose otoacariasis revealed their variability in diagnostic efficacy (Combarros *et al.*, 2019; Tyler *et al.*, 2020). The prevalence of the mite varies between the different populations studied e.g. indoor, stray and free-roaming cats (ElSeify *et al.*, 2016; Salant *et al.*, 2014; Montoya *et al.*, 2018; Coelho *et al.*, 2024; Riwu *et al.*, 2026).

The clinical importance of *O. cynotis* is underscored by the number of publications devoted to the issue of controlling it. In particular, for the treatment of ear-mite infestations in cats, there are various formulations of acaricides and topical combinations, including selamectin + sarolaner, fluralaner, tigolaner-containing combinations and esafoxolaner-based preparations (Becskei *et al.*, 2017; Bosco *et al.*, 2019; Taenzler *et al.*, 2017, 2018; Tielemans *et al.*, 2021; Blazejak, Cvejić, *et al.*, 2023; Blazejak, Viljoen, *et al.*, 2023; Yang & Huang, 2016). Consequently, the need for an accurate diagnosis should not be overlooked in order to choose the optimal treatment for an animal with ear mites.

Microscopic identification of *Otodectes cynotis* is necessary but not sufficient to describe the genetic relationship between isolates. Molecular markers: sequence comparison and phylogenetic analysis, in particular the mitochondrial cytochrome c oxidase subunit I (COI) gene The COI gene has also been used for genetic lineages and population structure of other parasitic mites from other regions of the world (Karp-Tatham *et al.*, 2020; Roy *et al.*, 2021; Nazarizadeh *et al.*, 2022). Mitochondrial COI DNA barcoding and nuclear markers have recently been used to investigate cryptic diversity of parasitic chiggers (Mohd-Redzuan *et al.*, 2026). Analysis of mitochondrial COI revealed that *Dermanyssus gallinae* from Tunisia was a separate lineage (Bettaieb *et al.*, 2027). These are not studies of *O. cynotis*. Nevertheless, the authors stress the importance of molecular techniques in the study of genetic diversity and evolutionary relationships of parasitic mites. Under a microscope but in molecular methods, genetic barcode similarity can be used along with conventional diagnostic techniques for identification of a particular strain. Universal primers for amplification of mitochondrial COI gene were developed by Folmer *et al.* (1994) and have been widely used in studies on genetic diversity of ectoparasitic mites (To study the population structure of the Psoroptidae family (Folmer *et al.*, 1994), ribosomal ITS2 and mitochondrial COI markers were used.

Previous molecular studies showed that *O. cynotis* isolates from different hosts and geographic regions may have a high overall sequence similarity with little variation in nucleotides. Several genetically related genotypes were identified in isolates from different hosts and geographic regions (Lohse *et al.*, 2002). The molecular characteristics of *O. cynotis* have been recently studied by COI sequencing and compared with GenBank records, sequence alignment and phylogenetic reconstruction (El-Dakhly *et al.*, 2025; Wilhelm *et al.*, 2025). Transcriptomic studies have also improved the molecular resources of the species (He *et al.*, 2022).

In recent years, molecular information on *O. cynotis* from Iraq has increased. Research in Babylon has focused on the prevalence and phylogenetic features of local isolates and recent work from Karbala has provided more molecular and sequencing data (Al-Khafaji & Al-Musawi, 2025a, 2025b; Mosa *et al.*, 2025; Alali *et al.*, 2026). The mite has been reported in domestic cats from several other areas of Iraq (Al-Tae & Al-Rubaie, 2018; Ghufran *et al.*, 2025; Khazal *et al.*, 2026). Nevertheless, molecular information from domestic cats in Mosul city remains limited. Generating local sequence data can therefore expand the geographical representation of *O. cynotis* in Iraqi and international sequence databases.

Otodectes cynotis is often diagnosed clinically and microscopically, but molecular characterization is an additional tool for confirming species identity and exploring genetic relationships among isolates. This is especially true when establishing reliable molecular reference data for geographically disparate

cat populations. Recent Iraqi investigations have enhanced knowledge of *O. cynotis* through molecular characterization in Babylon and sequencing-based analysis in Karbala, but more geographically representative data are valuable for understanding the distribution and genetic relationships of isolates across Iraq. In this context, the present study focuses on domestic cats in Mosul, northern Iraq, and combining microscopic examination with PCR targeting a partial fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Sequencing of selected PCR-positive samples and comparison with reference sequences provide molecular evidence for the identification of local isolates and contribute geographically specific sequence data to the available knowledge of *O. cynotis* in Iraq.
The current work was carried out to investigate *O. cynotis* in Mosul city on domestic cats by using conventional and molecular methods. Moreover, the research aimed to identify the parasite microscopically using PCR method directed to the partial sequence of mitochondrial COI gene and to study the genetic diversity of this parasite isolated from cats in Mosul city. In addition, the study aimed to analyze the genetic relationship between isolated strains and the most related sequences available in GenBank to contribute to the knowledge about the distribution and genetic variability of *O. cynotis* in Mosul city.

Materials and Methods

Study Animals and Sample Collection

The present study used 85 domestic female cats aged approximately 1–2 years. All were house cats, not strays. The study was conducted in Mosul City, Iraq, from 1 October 2025 to 30 April 2026. In the city's veterinary clinics, in the different residential areas, cats were examined. On clinical examination, an *Otodectes cynotis* infestation was revealed. Sterile swabs were used to collect secretions and crusts from the external auditory canal. Microscopic examination of samples for the presence and morphological identification of *O. cynotis*. Cats were included because they were referred to the participating veterinary clinics during the study period and samples were collected at different time points and analyzed in one laboratory. The study used female cats that were about 1 to 2 years old. The animals chosen were selected primarily because of the availability and accessibility of the samples and not because of any desire to study differences in susceptibility to *O. cynotis* between the sexes or age groups. Sample size was not formally calculated and was based on availability of eligible cats during the study period. The number of cats screened at each clinic was not recorded. *O. cynotis*-positive cats were examined by microscopic examination, and standard molecular PCR was performed.

Microscopic examination of ear swabs

Each swab was cultured on a clean glass slide, and a drop of distilled water was put on each slide for microscopic examination. Preparations were observed under a light microscope (objective 40×, eyepiece 10×) at a total magnification of ×400. Identification of adult mites was based on morphological characters, body shape, leg morphology, and mouthparts. *O. cynotis* was diagnosed in 12 (14.12%) of 85 examined cats by microscopic examination, whereas 73 (85.88%) cats were negative. Microscopy positive samples were chosen for further analysis by PCR. The microscopy-based prevalence was calculated as the proportion of cats with positive microscopic findings among the 85 examined cats. The 95% confidence interval was calculated using the Wilson score method.

Equipment and Consumables

The equipment and consumables used in the study are listed in (Table 1).

Table 1. Equipment and consumables used in the study.

No.	Equipment/Consumable	Manufacturer, Country
1	Eppendorf microcentrifuge	Wisd, Germany
2	Sensitive electronic balance	Want, China
3	Water bath	Gemmy, Taiwan
4	Microwave oven	LG, Korea
5	Vortex shaker	Thermo, USA
6	Thermal cycler (T100)	Bio-Rad, USA
7	Gel electrophoresis system	Bio-Rad, USA
8	Gel documentation system (Gel Doc EZ)	Bio-Rad, USA

9	1.5-mL Eppendorf tubes	Citotest, China
10	0.2-mL PCR tubes	Citotest, China
11	1000-µL pipette tips	Citotest, China
12	200-µL pipette tips	Citotest, China
13	10-µL pipette tips	Citotest, China

AddPrep Genomic DNA Extraction Kit from Tissue (Mini Kit) Kits and Reagents
The kits and reagents used in DNA extraction, PCR amplification, and agarose gel electrophoresis are presented below in (Table 2).

Table 2. Kits and reagents used in the molecular analysis.

No.	Kit/Reagent	Manufacturer, Country
1	AddPrep Genomic DNA Extraction Kit from Tissue (Mini Kit) Kits and Reagents	AddBio, Korea
2	AddBio 2× PCR Master Mix	AddBio, Korea
3	PCR-grade water	AddBio, Korea
4	100-bp DNA ladder (GeneDireX H3)	GeneDireX, Korea
5	GelRed nucleic acid stain	AddBio, Korea
6	Agarose	AddBio, Korea
7	10× TBE buffer	AddBio, Korea
8	Absolute ethanol	Scharlau, Spain

Molecular Detection of *Otodectes cynotis*
All 85 cats underwent microscopic examination for the detection of *Otodectes cynotis*. DNA extraction and PCR testing were subsequently performed only on the 12 samples that were positive by microscopic examination. Accordingly, PCR was used for molecular confirmation of microscopy-positive samples and was not performed on all 85 cats.

- DNA Extraction

Genomic DNA was extracted by using the Tissue Mini Kit (AddBio, Korea) AddPrep Genomic DNA Extraction Kit by following the manufacturer's instructions. Ear swabs were suspended in 300 micro-litre of sterile salt solution (0.9 percent NaCl) in a 1.5-mL Eppendorf tube and then 200 micro-L of lysis buffer plus 20 micro-L of proteinase K (20 mg/mL) were added. A mixture was incubated at 56 degrees C for an hour in a water bath and then 200 micro-L of binding buffer was added. Mixing with vortex was continued for 15 s to get a homogeneous suspension and incubated again at 56 degrees C for 10 min. After this, 200 micro-L of absolute ethanol was added and mixed again for 15 s. The mixture was transferred to a spin column inside of a 2-mL collection tube where it was centrifuged at 13000 RPM for 90 for second. The through-flow was discarded. The column was inserted into a new 2-mL collection, and 500 micro-L of Washing Solution 1 was centrifuged at 13,000 rpm for one min and then the flow-through was thrown away. Then 500 micro-L of Washing Solution 2 was added into it and centrifuged at 13000 rpm for one min, and the flow-through was once again thrown away. Centrifugation for a min at 13000 rpm will remove the residue, ethanol. The sample was transferred to a clean 1.5-mL microcentrifuge tube, and Elution solution was added. Here DNA was eluted after a 1-minute stand with 1 min-centrifuge at 13000 rpm. A re-hydration step was done with 100-micro Liter rehydration. The DNA preparations produced by extraction steps were stored at -20 Degrees C until molecular analytical studies further ahead. The extracted DNA was checked for quality and integrity using agarose gel electrophoresis techniques. A negative control for DNA extraction was run to avoid contamination during DNA extraction.

- Polymerase Chain Reaction (PCR)

PCR amplification was performed for a partial mitochondrial cytochrome c oxidase subunit I (COI) gene of *O. cynotis*. The LCO1490 and HC02198 primers were used for amplification. The primers were synthesized by Macrogen, Korea (Table 3).

Table 3. Primers used for amplification of the mitochondrial COI gene of *Otodectes cynotis*.

No.	Primer	Primer Sequence 5' – 3'	Product size	Reference
1	LCO1490	GGTCAACAAATCATAAAGATATTGG	~700	(Folmer *et al.*, 1994)
2	HC02198	TAAACTTCAGGGTGACCAAAAAATCA		

The PCR reactions were performed in 25 µL of AddBio 2× PCR Master Mix (AddBio, Korea). Each reaction consisted of 12.5 µL of 2× Master Mix, 6.5 µL of PCR grade water, 1 µL of forward primer (10 µM), 1 µL of reverse primer (10 µM) and 4 µL of DNA template (40 ng/µL).
PCR amplification was performed with a T100 Thermal Cycler (Bio-Rad, USA) using the cycling conditions described in (Table 4).

Table 4. PCR cycling conditions for amplification of the mitochondrial COI gene of *Otodectes cynotis*.

No.	Step	Temperature	Time	Cycle
1	Initial polymerase activation	95°C	10 min	1
2	Denaturation	95°C	45 s	35
3	Annealing	55°C	45 s	35
4	Extension	72°C	1 min	35
5	Final extension	72°C	5 min	1
6	Hold	4°C	∞	—

To monitor the possible contamination, we included a negative control in the PCR assay. No amplification was detected in the negative control. The tested samples generated the expected amplicon of around 700 bp.

- **Agarose Gel Electrophoresis**

The PCR product was further analyzed in 1.5% agarose gel with 1× TBE buffer. For visualization of the DNA bands the PCR products were diluted 1:2 in 4 µl of GelRed staining reagent. The PCR product samples were loaded in the wells in 7:1 ratio and separated by electrophoresis at 75 V for 1 h in horizontal electrophoresis chamber (1× TBE buffer, Bio-Rad system). The molecular size marker was 100 bp DNA ladder (GeneDireX H3, Korea). Images were taken under UV light with the help of Gel Doc EZ Gel Documentation System (Bio-Rad, USA) and stored images were taken for further analysis.

- **DNA Sequencing**

Selected polymerase chain reaction (PCR) products exhibiting the expected amplification pattern for DNA sequencing, along with corresponding COI primers, were sent to Macrogen in Korea for DNA sequencing. The resulting nucleotide sequences were received as transcripts and subsequently analyzed to confirm the molecular identity of the parasite and to assess the genetic relationships between the isolates. Sequencing was restricted to three PCR-positive samples due to financial and resource limitations. Selection was also made based on technical considerations related to the quality of the PCR products for sequencing. To examine genetic relationships of the examined specimens of *Otodectes cynotis*, the obtained sequences were compared with reference sequences from the GenBank database.

- **Sequence Alignment and Nucleotide Variation Analysis**

In this study, three COI sequences (GenBank accession numbers PZ715811, PZ715812, and PZ715813) were compared with six reference sequences of *Otodectes cynotis* from GenBank (KP676688, KP676678, KP676679, KP676687, KP676680, and MW350132). Sequences were aligned using Clustal Omega version 1.2.4. Nucleotide diversity of the aligned sequences was studied. The resulting alignment was used to assess the sequence length, variable nucleotide sites, and parsimony informative sites.

- **Sequence and Phylogenetic Analysis**

Homologous sequences available in the NCBI GenBank database were compared with the partial nucleotide sequences of the COI gene obtained in this study using the BLASTn algorithm. Pairwise comparisons were made using query coverage and nucleotide identity. The three local sequences of *Otodectes cynotis* (PZ715811, PZ715812, and PZ715813) were aligned with the six reference sequences obtained from GenBank (KP676688.1, KP676678.1, KP676679.1, KP676687.1, KP676680.1, and MW350132.1) using the Clustal Omega version 1.2.4. The query sequences were selected based on the highest BLASTn similarity, query coverage, and availability of the corresponding partial COI region. An analysis of the alignment for nucleotide variation was performed to identify variable and parsimony informative sites.Phylogenetic relationships were inferred by the Maximum Likelihood (ML) method

based on the Tamura-Nei model [53] in MEGA version 11 [54]. This model allows for varying transition rates and nucleotide frequencies. Bootstrap support for the branches was tested with 100 replicates. The phylogenetic tree was rooted with the outgroup species *Trombidium holosericeum* (GenBank accession number MK995460.1) in an effort to separate the *O. cynotis* ingroup from the outgroup taxon.

Results

The presence of *Otodectes cynotis* was detected by microscopic examination in 12 of the 85 examined cats, corresponding to a prevalence of 14.12% (95% CI: 8.26%–23.07%). PCR amplification of the expected approximately 700-bp COI fragment was subsequently obtained from all 12 microscopy-positive samples tested (12/12, 100%). Since PCR testing was restricted to microscopy-positive samples, these findings represent molecular confirmation of the selected positive samples rather than an independent PCR-based prevalence estimate for all 85 cats (Fig. 1).

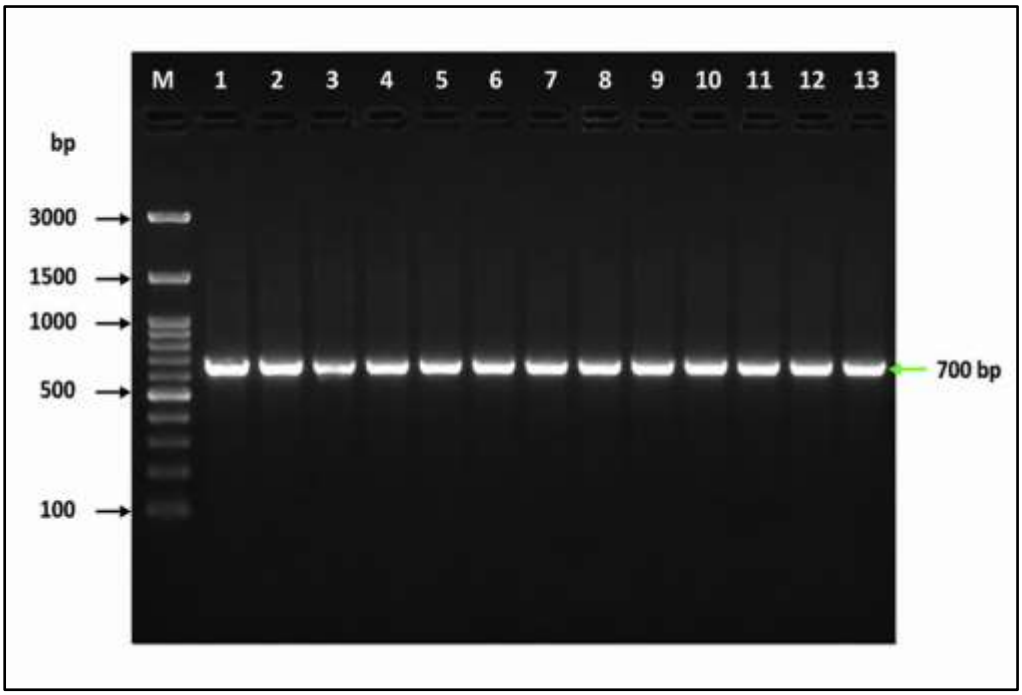

Figure 1. PCR amplification of the partial mitochondrial COI gene of *Otodectes cynotis*. Lane M: 100-bp DNA ladder; lanes 1–12: positive samples; lane 13: negative control.

DNA Sequencing and GenBank Accession Numbers

Three PCR-positive specimens were sequenced for DNA. The partial COI sequences obtained were approximately 654 bp long. The three local sequences were submitted to NCBI GenBank database under accession numbers PZ715811, PZ715812 and PZ715813. The sequences were used for comparative sequence and phylogenetic analysis.

BLASTn Analysis

The BLASTn analysis showed 100% query coverage for the compared local sequences. The nucleotide identities to the reference *O. cynotis* sequences were from 99.39% to 100%. The sequences were KP676688, KP676678, KP676679, KP676687, KP676680 and MW350132. They were found to be *O. cynotis* sequences with high similarity in the database. The results gave molecular evidence for identification of the local isolates as *O. cynotis* (Table 5).

Sequence Variation Analysis

Multiple sequence alignment included nine COI sequences, comprising three sequences generated in the present study and six reference sequences retrieved from GenBank. The sequence lengths reported in the alignment ranged from 652 to 654 nucleotides. Five variable nucleotide sites were identified in the alignment, of which three were parsimony-informative sites and two were singleton variable sites.

Table 5. BLASTn comparison of local *Otodectes cynotis* COI sequences with reference sequences retrieved from GenBank, including query coverage, nucleotide identity, GenBank accession numbers, and country of origin.

Sample	Parasite Identified	Query	Identical	GenBank Accession	Country
PZ715811 PZ715812 PZ715813	*Otodectes cynotis*	100	100	KP676688	China
		100	99.69	KP676678	China
		100	99.69	KP676679	China
		100	99.54	KP676687	China
		100	99.54	KP676680	China
		100	99.39	MW350132	China

Multiple Sequence Alignment and Nucleotide Variation

The multiple sequence alignment of the partial mitochondrial COI sequences showed a high level of conservation of the nucleotides between the three local isolates and the reference sequences of *O. cynotis*. The substitutions of the nucleotides in the aligned region were limited to a few positions. The analyzed sequences were approximately 654 bp long, and they showed a high sequence similarity to the reference sequences and the local isolates PZ715811, PZ715812 and PZ715813. The length of the reference sequence MW350132 in the alignment was about 652 bp (Fig. 2).

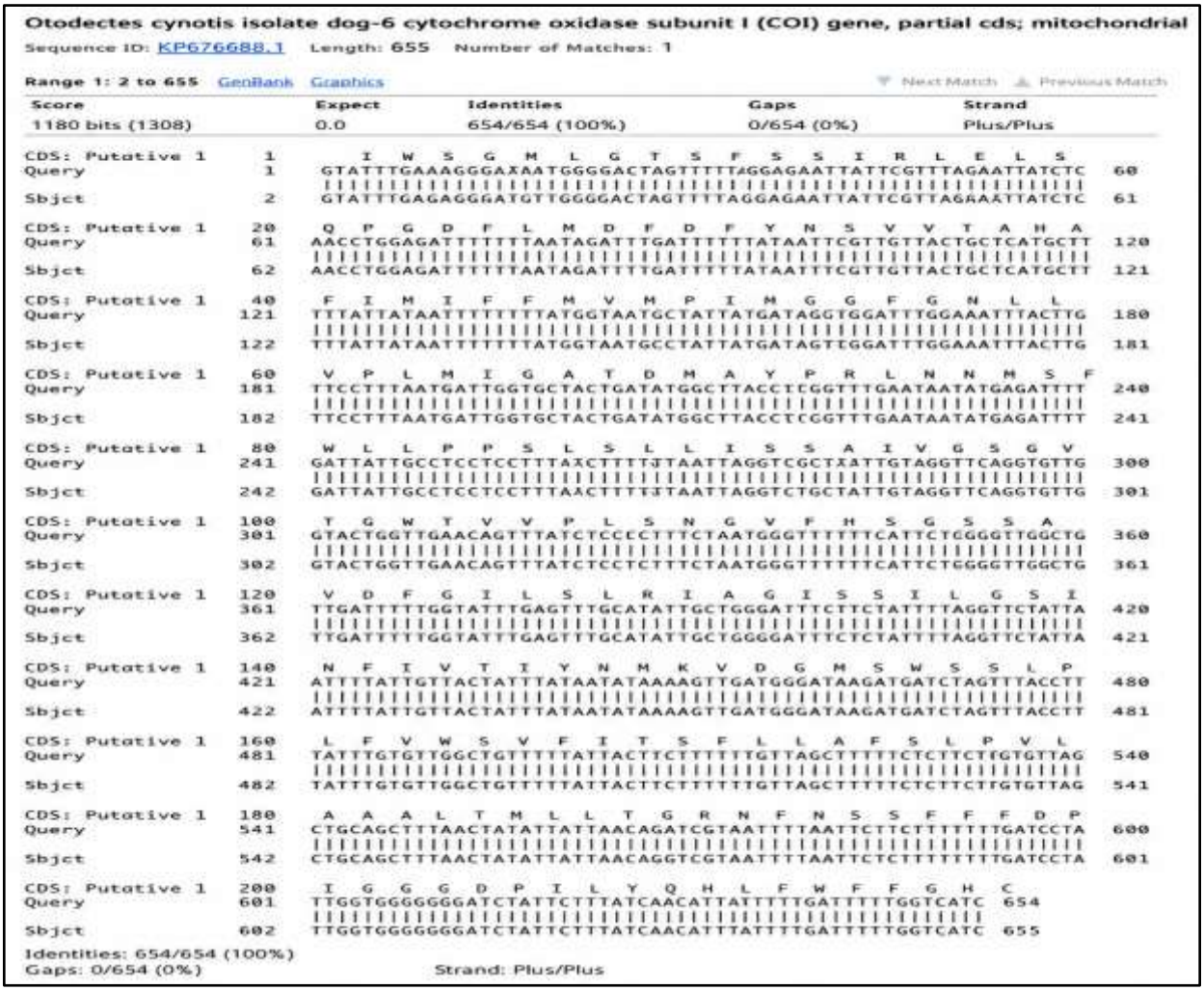

Figure 2. Multiple sequence alignment of partial mitochondrial COI gene sequences of *Otodectes cynotis* isolates obtained in the present study and selected reference sequences retrieved from GenBank.

Phylogenetic Analysis

The phylogenetic tree was constructed with the Maximum Likelihood method based on the Tamura–Nei model in MEGA version 11 with a bootstrap of 100 replications. The three local isolates, PZ715811, PZ715812, and PZ715813, were positioned within the main *O. cynotis* phylogenetic group together with the reference sequences. The *O. cynotis* group was separated from the outgroup *Trombidium holosericeum* (MK995460.1) with a bootstrap support value of 96%. Major internal nodes within the *O. cynotis* group showed bootstrap support values of 100%. The phylogenetic position of the local isolates was similar to the high nucleotide similarity observed in BLASTn analysis. The maximum likelihood phylogenetic tree showed the three local COI sequences (PZ715811, PZ715812, and PZ715813) in the same clade as the main *Otodectes cynotis*, together with the six reference sequences retrieved from GenBank. The *O. cynotis* group was separated from the outgroup *Trombidium holosericeum* (MK995460.1) with 96% bootstrap support. Major internal nodes within the *O. cynotis* group received 100% bootstrap support. These results were consistent with the high nucleotide identity observed in the BLASTn analysis (Fig. 3).

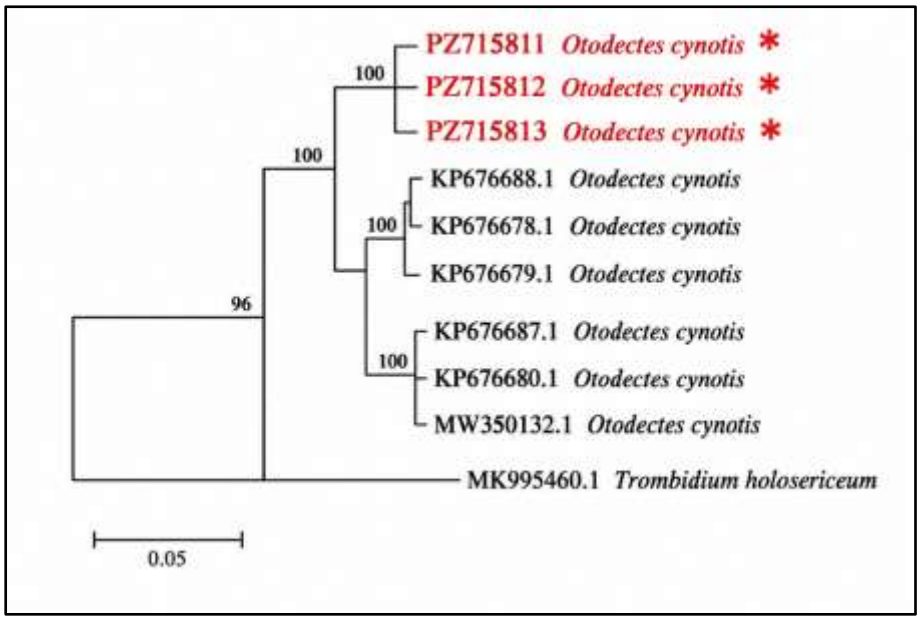

Figure 3. Maximum Likelihood (ML) phylogenetic tree based on partial mitochondrial cytochrome c oxidase subunit I (COI) gene sequences, showing the relationships between the three *Otodectes cynotis* isolates obtained in the present study (PZ715811, PZ715812, and PZ715813; marked with red asterisks) and six reference sequences retrieved from GenBank (KP676688.1, KP676678.1, KP676679.

1, KP676687.1, KP676680.1, and MW350132.1). *Trombidium holosericeum* (MK995460.1) was used as the outgroup. The tree was constructed in MEGA version 11 using the Tamura–Nei model with 100 bootstrap replicates. Bootstrap support values are indicated at the corresponding nodes.

Discussion

The present study showed that *Otodectes cynotis* was detected in 12 out of 85 domestic female cats (14.12%) in Mosul city. This indicates the presence of ear mites in cats in the houses of the study area. This rate was lower than some rates reported in Iraqi cat populations such as in Babylon and Karbala (Ghufran *et al.*, 2025; Al-Khafaji Al-Musawi, 2025b). Furthermore, it was lower than the rate that was recently found for domestic cats in an urban population in Mataram and those that were presented to veterinary clinics in Istanbul (Deniz *et al.*, 2026; Riwu *et al.*, 2026). Variation in the study may be due to variation in host populations, housing conditions, geographic location, season, sample size, diagnostic techniques. Care should be taken when comparing populations, especially stray and pet cats (ElSeify *et al.*, 2016; Salant *et al.*, 2014).

The clinical significance of *O. cynotis* is well known as it is associated with abnormalities of the external ear canal and can aggravate feline otitis externa (Coelho *et al.*, 2024; Tyler *et al.*, 2020). Management has to be properly diagnosed. Several acaricidal treatments have been effective against the mite, but treatment efficacy was not the aim of the present study (Becskei *et al.*, 2017; Bosco *et al.*, 2019; Taenzler

et al., 2017, 2018; Tielemans *et al.*, 2021; Blazejak, Cvejić, *et al.*, 2023; Blazejak, Viljoen, *et al.*, 2023; Yang & Huang, 2016).

Molecular results further support the identification of parasites. The positive samples showed the expected result of amplification of the partial mitochondrial COI gene. In psoroptid mites, the COI marker has been previously used and the sequencing of the COI has been recently reported as successful for *O. cynotis* (Folmer *et al.* 1994; Juan *et al.* 2015; El-Dakhly *et al.* 2025; Wilhelm *et al.* 2025). The agreement of the PCR results with the parasitological identification confirms the need to associate the morphological and molecular techniques.

The three local COI sequences (PZ715811, PZ715812, and PZ715813) had 100% query coverages and 99.39–100% nucleotide identities with the reference sequences of *Otodectes cynotis* used in the BLASTn. The results agree with the molecular identification of local isolates as *O. cynotis* and show high similarity in the sequence of the analyzed fragment of COI. Also, high COI sequence identities were observed between the obtained sequences and reference sequences available from GenBank in previous molecular studies on *O. cynotis* (El-Dakhly *et al.*, 2025; Wilhelm *et al.*, 2025).However, high nucleotide identity of a partial COI sequence should not be seen as a direct measure of genetic diversity in the population or as evidence of genetic homogeneity. Three PCR-positive samples from the current study were successfully sequenced, and the obtained sequences represent a small subset of the examined cats and are not representative to describe the genetic diversity or the population structure of *O. cynotis* in Mosul. No clear separation of the ITS2 genotypes was observed based on host species or geographic distribution; however, similar genotypes have been previously identified in Otodectes isolates from different hosts and geographic areas (Lohse *et al.*, 2002). Similarly, RAPD-PCR analysis showed genetic variation between isolates of *O. cynotis* from cats and dogs (Salib and Baraka, 2011). Results of these studies are instructive but cannot be directly compared with the COI results of the present study because of different molecular markers and analytical approaches.The ML tree placed the three local sequences inside the main *O. cynotis* clade, supporting their molecular identification. The comparison of the different genetic markers in *O. cynotis* showed that the phylogenetic topologies can be different depending on the gene studied (Al-Khafaji and Al-Musawi, 2025a). The tree obtained here gives support for the taxonomical position of the analyzed COI sequences but not a complete picture of the population relationships. Although some reference sequences used in this analysis were from China, the similarity to the Mosul sequences does not suggest a geographic relation, a common source, or a route of transmission. Such conclusions would require more sequences from other sites and a dedicated population-genetic analysis.

Mitochondrial COI has been useful for studies of genetic relationships between parasitic mites, e.g., *Dermanyssus gallinae* and *Laelaps agilis*. The studies have shown that the analysis of COI sequences can identify the phylogenetic lineages and geographic patterns of genetic variation in mite populations (Karp-Tatham *et al.*, 2020; Nazarizadeh *et al.*, 2022; Bettaieb *et al.*, 2026). Likewise, nuclear DNA barcoding has revealed cryptic diversity in parasitic chiggers (Mohd-Redzuan *et al.*, 2026) and broader reviews of *D. gallinae* have highlighted the benefits of employing genetic data in studies of population structure (Roy *et al.*, 2021). These results give a wider methodological context to the present COI analysis. However, the present results should be considered as preliminary molecular evidence and not a complete estimation of local population genetic diversity, as only three *O. cynotis* isolates from Mosul were sequenced.

The current study adds molecular data from Mosul city to the growing Iraqi dataset. Recent data from Babylon and Karbala have provided epidemiological, molecular and phylogenetic data on *O. cynotis* (Mosa *et al.*, 2025; Al-Khafaji & Al-Musawi, 2025a, 2025b; Alali *et al.*, 2026). The parasite was found in domestic cats from various regions in other Iraqi studies (Al-Tae & Al-Rubaie, 2018; Ghufran *et al.*, 2025; Khazal *et al.*, 2026). Thus, the three sequences generated in the present work contribute to the available geographic representation of *O. cynotis* and provide locally sourced molecular records for future comparative studies. Broader molecular resources developed because of transcriptomic work may also aid future studies of this parasite (He *et al.*, 2022).

A major limitation of this study is that all cats examined were female and approximately 1–2 years of age, a decision mainly dictated by sample availability and ease of acquisition. Therefore, the prevalence of *Otodectes cynotis* found (14.12%) should be interpreted according to the studied population and should not be considered representative of all domestic cats in Mosul. Furthermore, this limitation in the sample size did not allow us to compare infestation rates by sex or age groups. Future studies should include male and female cats of a wider age range and a more representative sampling strategy to allow for a more generalizable estimate of prevalence in the local cat population.

The restricted number of sequenced samples represents an important limitation of the present study.

Only three PCR-positive samples were sequenced due to financial and resource constraints and technical problems concerning the quality of PCR products. The obtained sequences were compared with the reference sequences in GenBank. However, three sequences are not enough to describe in detail the genetic diversity of *Otodectes cynotis* in Mosul. Therefore, phylogenetic results should be taken as preliminary evidence of genetic relationships and not as a representative assessment of genetic diversity in the population. Further studies of a larger number of sequenced specimens from several locations and host populations are needed to provide a more complete picture of genetic variation in this parasite.

Conclusions

The current study reports the prevalence of *Otodectes cynotis* in domestic cats in Mosul, Iraq. Microscopy identified the mite in 12/85 cats (14.12%, 95% confidence interval: 8.26%–23.07%), and all 12 microscopy-positive samples were confirmed by PCR. Partial sequences of mitochondrial COI from local samples were deposited in GenBank under the accession numbers PZ715811, PZ715812, and PZ715813. The molecular identification was confirmed by the high nucleotide similarity of the local isolates with the reference sequences, but the low number of sequenced samples could not provide a complete characterization of the genetic diversity or population structure of *O. cynotis* in Mosul. Further studies involving more isolates from male and female cats, different age groups, and different areas in Mosul and surrounding areas are needed to better characterize the genetic diversity and population structure of this parasite.

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No conflict of interest relevant to this article was reported.

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The study was reviewed and approved by the Scientific and Ethical Committee at the College of Science, University of Mosul.

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