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Original Research Article

Using the COX Gene (COI) for Molecular Identification of Earthworms Collected from Selected Areas in Al-Muthanna Province, Iraq

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Abstract: In this study, earthworms were collected from eight selected locations within Samawah City, Al-Muthanna Province, southern Iraq. Specimens were collected from September 2024 to August 2025 and identified using morphological and molecular methods. The mitochondrial COX1 gene (710 bp) was sequenced for molecular confirmation. four species belonging to two families (Lumbricidae and Megascolecidae) were identified: Aporrectodea trapezoides, Aporrectodea rosea, Imetacolex partiarchalis and Amynthas corticis. The presence of these species in arid southern Iraq highlights the role of human-mediated dispersal and irrigation agriculture in soil fauna distribution. This study contributes to the poorly documented earthworm fauna of Iraq and underscores the need for further biodiversity surveys in the region.

Keywords: Earthworms, Molecular and Morphological identification, Samawah, Iraq.

INTRODUCTION

Iraq is a Middle Eastern country with diverse topography including the Tigris and Euphrates river basins, alluvial plains, marshlands, western deserts and northern mountainous regions. The climate ranges from arid in the southwest to continental in the north, (Jabr, 2019). Despite its varied ecosystems, soil biodiversity—particularly earthworm fauna—has been largely overlooked in scientific literature. Previous studies have been sporadic, localized, and often limited. The first records of Iraqi earthworms date back to regional faunal surveys in the mid-20th century (Othman and Taeb Ahmad, 2020), with more recent studies focusing on Baghdad and the Kurdistan region (Kamal *et al.,*2023). However, no comprehensive national checklist exists, and molecular data are scarce. This study aims to provide updated records from southern governorates such as Al-Muthanna, and to establish the first records of earthworms in southern Iraq using integrative taxonomic approaches.

MATERIALS AND METHODS

Samawah City is located in Al-Muthanna Province, southern Iraq, along the Euphrates River. The climate is hot and arid, with average summer temperatures exceeding 45°C and low annual rainfall (<150 mm) (Kadhim, 2024). Sampling was conducted in eight locations representing agricultural, grassland, and urban garden habitats (Table 1).

2.1. Specimen Collection

Earthworms were collected from September 2024 to August 2025 by digging and hand sorting. A total of 14 adult specimens were collected. Specimens were anaesthetized in 15% ethanol and fixed in 80% ethanol for morphological and molecular analysis.

2.2. Morphological Identification

Specimens were examined under a stereomicroscope.

Identification was based on clitellum position, setae arrangement and tubercula pubertatis following keys by Sims & Gerard (2023) and Csuzdi & Zicsi (2003).

2.3. Molecular Analysis

Genomic DNA was extracted from muscle tissue using the WizPrep™ gDNA Mini Kit. The COX1 gene was amplified using Folmer primers LCO1490 and HCO2198. Genetic analysis was performed on polymerase chain reaction (PCR) products derived from (14) PCR samples targeting specific genomic regions of the mitochondrial COI gene, provided by CD Genomics, USA. The sequencing process utilized high-throughput Applied Biosystems 3730xl DNA Analyzers based on capillary electrophoresis, which determine the precise nucleotide sequence of each DNA fragment through fluorescent dye-terminator chemistry. Sequences were compared with GenBank using BLASTn.

Table 1. Sampling locations and habitat types in Samawah City, Iraq.

No. of Sampling Site	Locality Name	Latitude (N)	Longitude (E)	Species	Habitat
1	Al-fazaya	31.26663	45.04998	*Apporrectodea trapezoides*	Farm land
2	Al-helal	31.5860	45.2220	*Apporrectodea trapezoides*	Farm land
3	Al-najme	31.66854	45.18207	*Amynthas corticis*	**Palm groves**
4	Al-majd	31.32111	45.21917	*Apporrectodea rosea*	Agricultural land
5	Abu-shrish	31.317	45.283	*Apporrectodea trapezoides*	Agricultural land
6	Al-rmaitha	31.52908	45.21091	*Imetescolex patriarchalis*	Anthropogenic
7	Al-warkaa	31.47019	45.29085	*Imetescolex patriarchalis*	Wet land
8	Al-samawa	31.3188	45.2806	*Apporrectodea trapezoides*	Anthropogenic urban garden

RESULTS

Four species belonging to two families were identified across different sampling stations.

3.1. Morphological Description

Family: Lumbricidae

***Aporrectodea trapezoides* (Dugès, 1828)**

Clitellum and tubercula respectively on segments 27-34, 31-33. Tubercule tripartite, dorsal surface light to dark brown, 100-180 segments. Fig1 A.

***Aporrectodea rosea* (Savigny, 1826)**

Clitellum on segment 26-31, tubercles on segments 29-31. Dorsal pores are readily visible, especially on the clitellum, 100-150 segments. Fig1 B.

***Imetacolex patriarchalis* (Rosa, 1893)**

Diagnosis: Clitellum extends on segments (21), 22, 23–33, 34 saddle-shaped. Tubercles usually346 on ½30–½33. Male pore on 15, great slit. Fig1 C.

Family: Megascolecidae

***Amynthas corticis* (Kinberg, 1867)**

The clitellum is annular and extends over segments 14–16. The male pores are present as one pair on the ventrolateral surface of segment 18, and the female pore is single, located on the mid-ventral line of segment 14. The setal arrangement is perichaetine. Fig1 D.

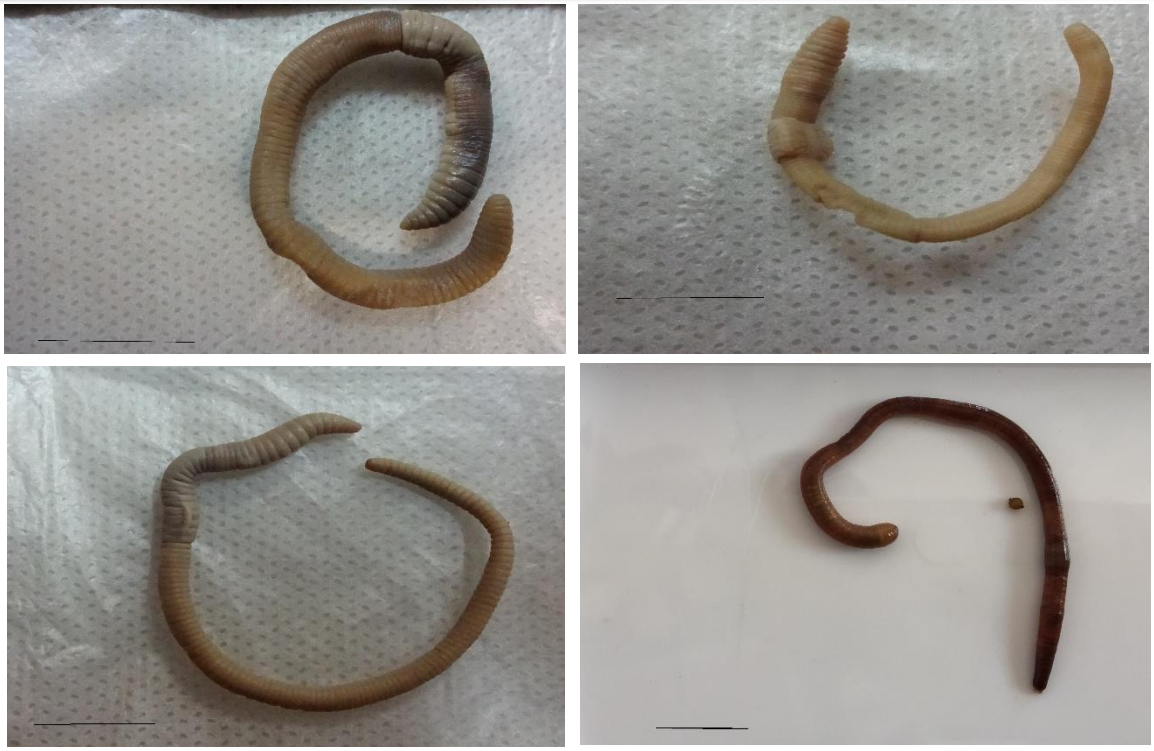


Figure1. Different species identified in the present study. A: *Apporrectodea trapezoids*; B: *Apporrectodea rosea*; C: *Imetescolex patriarchalis*; D: *Amynthas cortices*. All figures are at a scale of 1 cm.

3.2. Molecular Identification

DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COX1) gene (710 bp) was employed to confirm the morphological identifications. The resulting sequences were deposited in GenBank under accession numbers: (PX788612 , PX788613, PX788614 , PX788615 , PX788616, PX788617, PX788618, PX788619 , PX788620, PX788621 ,PX788622, PX788643, PX788644, PX788645).

PCR technique targeting mitochondrial *COX-I* gene in all genomic DNA extracted samples from 14 earthworms sample (Figure 2). A single fragment of 710 bp length was successfully amplified using primer of mitochondrial *COX-I* gene when separated by 1.5 % agarose gel electrophoresis.

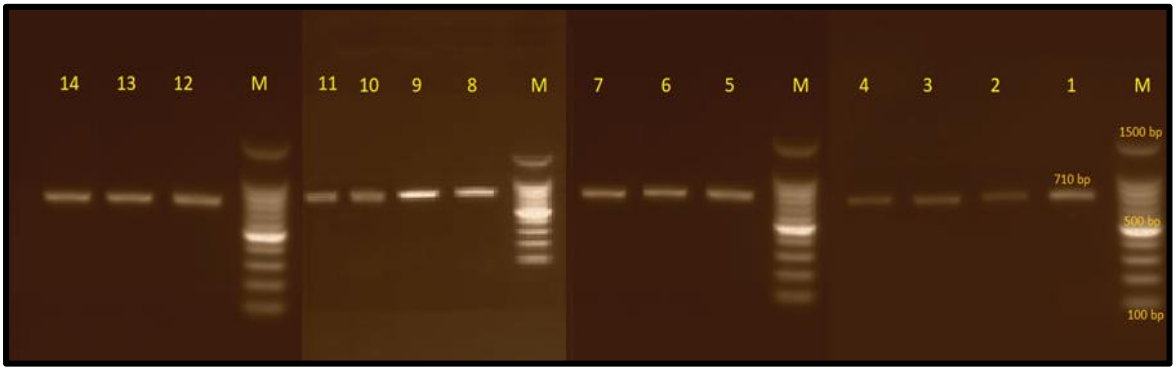


Figure 2: Amplification of mitochondrial cytochrome c oxidase subunit I from 14 different samples of earthworms in lanes 1-14. PCR products were 710 bp and electrophoretically migrated at 70 volts for 1 hour on 1% agarose gel using a 100 bp DNA ladder in lane M.

3.3. Molecular Analysis

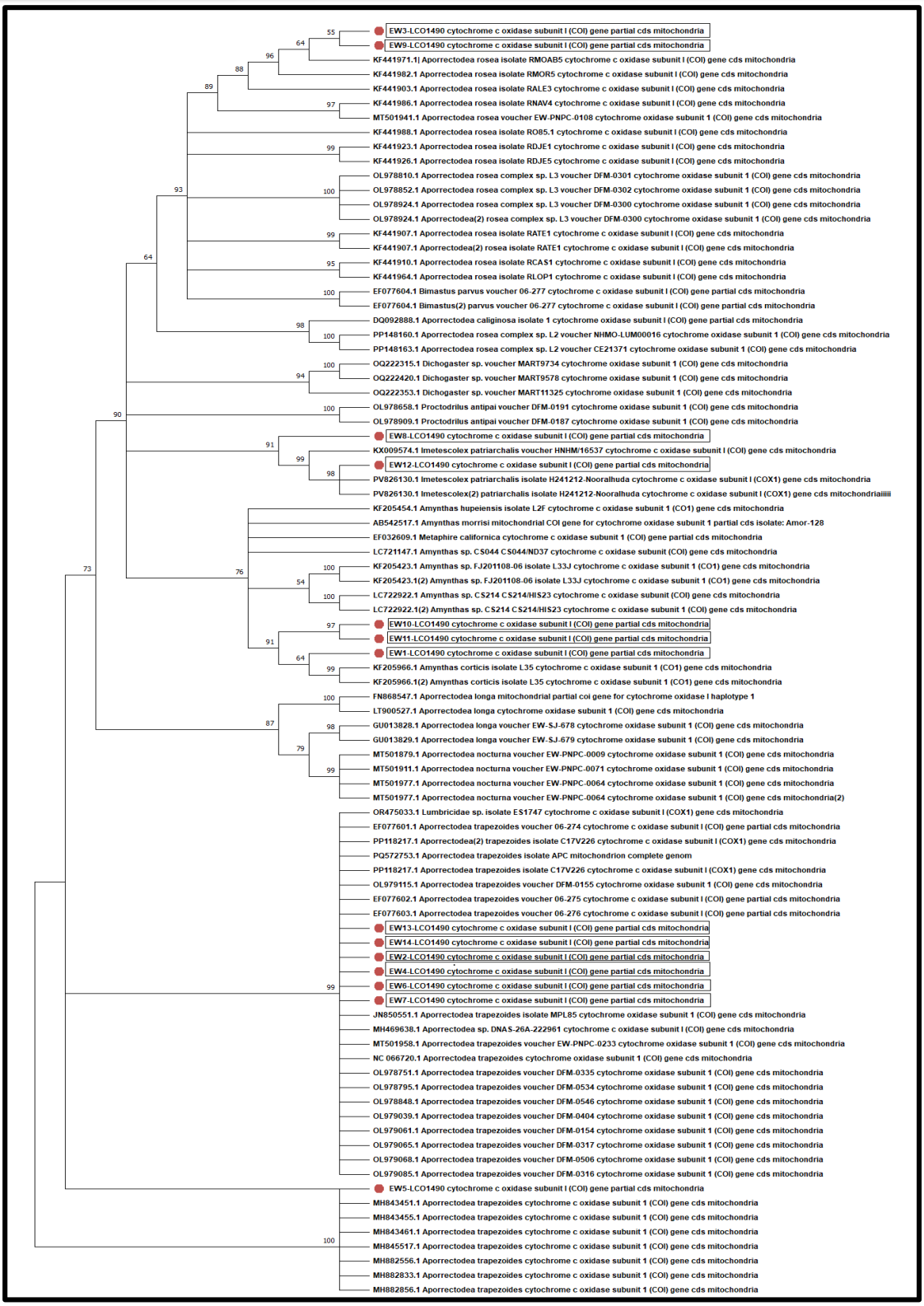
Partial mtCOX1 gene sequences (710 bp) of 14 PCR products from earthworm samples were sequenced in this study. The results of the mtCOX1 gene sequencing analysis identified three samples belonging to the genus *Amynthas corticis*, seven samples belonging to the genus *Aporrectodea trapezoides*, and two samples each from *Aporrectodea rosea* and *Imetescolex patriarchalis* using BLASTn multiple alignment with available sequences of earthworm species recorded in the GenBank database from different countries (Table 2).

Table 2. *mtCOX1* gene sequences of earthworm species from the present study and their closest matches in the GenBank database.

Recorded Species in this study	% similarity	Country	Accession numbers
Amynthas corticis	98%	China	KF205966.1
Amynthas corticis	92%	China	KF205966.1
Amynthas corticis	90%	China	KF205966.1
Aporrectodea trapezoides	99%	China	NC_066720.1
Aporrectodea trapezoides	99%	China	NC_066720.1
Aporrectodea trapezoides	99%	China	NC_066720.1
Aporrectodea trapezoides	99%	China	NC_066720.1
Aporrectodea trapezoides	99%	China	NC_066720.1
Aporrectodea trapezoides	99%	China	NC_066720.1
Aporrectodea trapezoides	99%	China	NC_066720.1
Aporrectodea rosea	99%	Spain	KF441982.1
Aporrectodea rosea	99%	Spain	KF441982.1
Imetescolex patriarchalis	96%	Turkey	KX009574.1
Imetescolex patriarchalis	96%	Turkey	KX009574.1

3.4. Phylogenetic Analysis Phylogenetic trees were constructed using COX1 gene sequences of earthworm species identified in this study, along with reference sequences retrieved from the GenBank database. The analysis revealed distinct phylogenetic relationships among the taxa.

Samples of *Aporrectodea rosea* from Iraq clustered closely with reference sequences of the same species from Spain (KF441971.1, KF441982.1). Specimens of *Metapheretima patriarchalis* (previously referred to as *Imetacolex patriarchalis*) formed a separate clade and showed 96% sequence identity to conspecific sequences from Turkey (KX009574.1) and a recently recorded sequence from Iraq (PV826130.1). Three specimens were identified as *Amynthas corticis* based on their close relationship with sequences from China (KF205966.1). Finally, seven specimens of *Aporrectodea trapezoides* grouped with high similarity (99%) to reference sequences from China (NC_066720.1, EF077603.1), France (OL979039.1), and other regions, as illustrated in Figure 3.



Figur 3. Phylogenetic analysis of COX-1 gene sequences from earthworm species in this study and related sequences from the GenBank database.

DISCUSSION

This study presents the first records of several earthworm species in southern Iraq. An integrative approach was used to document the distribution of earthworms in this region, confirming the presence of four species. Molecular analysis was particularly valuable for identifying *Imetacolex patriarchalis*, as this species is sexually mature for only a short period annually, a factor that has historically led to the description of several synonymous names (Szederjesi

et al., 2014).

A. rosea is a peregrine species widely distributed in Europe and western Asia, often associated with agricultural and grassland soils. Its presence in Samawah is likely due to human-mediated introduction via agricultural activities. This species inhabits various soil types, including soils under bushes and the banks of water courses. It is well-adapted to cultivated land, a finding supported by Piearce (1978), who demonstrated that *Ap. rosea* typically consumes well-degraded amorphous plant material. The morphological measurements of *A. rosea* specimens from Samawah are consistent with those reported from Iran and Turkey (Latif et al., 2025).

Aporrectodea trapezoides was found in agricultural and other anthropogenic sites. *Imetacolex patriarchalis* is a lesser-known species, previously recorded in Turkey and Iran. Its occurrence in Iraq suggests a broader distribution within the Mesopotamian region than previously recognized. The lower genetic sequence similarity (96%) with Turkish specimens may indicate genetic divergence due to geographic isolation or local adaptation. This species prefers soil with abundant organic material and is abundant in Northern Turkey and common in the Caucasus (Pavlíček & Csuzdi, 2006). Its known range extends west to Karpathos Island (Szederjesi, 2015). The high mitochondrial COX1 gene sequence similarity (99%) of *Aporrectodea trapezoides* and *A. rosea* from southern Iraq with populations from Europe and West Asia provides strong molecular evidence for their status as peregrine (widely introduced) earthworm species. This pattern is characteristic of species dispersed via human-mediated pathways, particularly agricultural activity. The movement of plants, soil, and agricultural machinery has historically served as a primary vector for earthworm introduction beyond their native ranges (Hendrix et al., 2008). Both *A. trapezoides* and *A. rosea* are well-documented cosmopolitan species often associated with cultivated land, and their global distributions largely mirror patterns of human settlement and agriculture (Blakemore, 2002; Pérez-Losada et al., 2005). The genetic homogeneity observed across vast geographical distances—from Iraq to Spain and China—indicates recent and repeated introductions that have not allowed for substantial genetic divergence, a hallmark of species spread by human activity (Rougerie et al., 2009). Furthermore, the arid climate of southern Iraq is not a presumed historical barrier for these temperate-adapted species, making natural dispersal unlikely and reinforcing the hypothesis of anthropogenic introduction. These molecular findings align with global studies on earthworm biogeography, where high genetic similarity across continents frequently signals human-aided dispersal rather than natural range expansion (James & Hendrix, 2004). Also The confirmed presence of *Amynthas corticis* in southern Iraq, based on a 98–99% match to East Asian references (KF205966.1, China), provides strong molecular evidence of a significant human-mediated introduction. This species belongs to a genus (*Amynthas*) notorious for its peregrine status and invasive potential, particularly through the global trade in horticultural plants, soil, and compost (Chang et al., 2016; Snyder et al., 2019). This study indicates that the presence of three of the four identified earthworm species in southern Iraq (*Aporrectodea trapezoides*, *A. rosea*, and *Amynthas corticis*) strongly aligns with a pattern of human-mediated introduction via agricultural activities and plant trade. In contrast, *Imetacolex patriarchalis* may represent a native population with a broader distribution in the Mesopotamian region, showing genetic divergence indicative of geographic isolation.

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