GENETIC DIVERSITY IN LUMBRICIDAE AND MEGASCOLECIDAE (OLIGOCHAETA) INFERRED FROM MITOCHONDRIAL DNA, WITH DESCRIPTION OF A NEW *Aporrectodea longa* AND NOTES ON *Pithemera bicincta*

N. M. S. Othman*,[1], S. T. Ahmad[2] and S. H. Sedo[1]

[1]Department of Biology, Faculty of Science, University of Zakho, Kurdistan Region, Iraq
[2]Department of Biology, College of Science, University of Salahaddin - Erbil, Kurdistan Region, Iraq
*Corresponding Author's Email: naz.amr2013@gmail.com

ABSTRACT

Identification at a species level is one of the primary issues in earthworm species biodiversity, which restricts appropriate measurement of community structure and conservation level. Morphological characters together with molecular status, especially mitochondrial DNA markers have been useful to solve the ambiguous earthworm's diversity. In this study, we examined the genetic diversity and phylogeny of earthworms in the Kurdistan Region of northern Iraq with reference to the mitochondrial cytochrome c oxidase subunit I (*Cox1*) gene. Between September 2024 and August 2025, a total of 200 earthworms were collected in various localities. Mitochondrial DNA was obtained using gizzard tissue, and a 523 bp fragment of *Cox1* gene was amplified and sequenced. Three lumbricid species were identified through molecular identification that included: *Aporrectodea trapezoides*, *Aporrectodea longa*, and *Pithemera bicincta*. Maximum Likelihood method of phylogenetic analysis resulted in resolution of two major clades with the Iraqi isolates falling in the same clade as the Asian and European reference sequences. The haplotype network analysis revealed five haplotypes Hap_1-Hap_5 with Hap_1 being the most widespread in the areas of Iraq, Europe, Asia, and North America, which indicates extensive dispersal and high genetic connectivity. The haplotype diversity was moderate (Hd = 0.39), whereas the nucleotide diversity was high (π = 0.10), meaning that there was a significant sequence divergence between the taxa analyzed. In terms of neutrality tests, Tajima D was negative (-1.59) that implies the existence of more rare alleles that could be connected to the demographic growth or selection. Our results represent the initial molecular validation of *P. bicincta* in Iraq and expand the known range of *A. longa*. In general, the results provide novel molecular information on earthworm's diversity in Iraq.

Keywords: Earthworms; *Aporrectodea trapezoides*; *Aporrectodea longa*; *Pithemera bicincta*; *Cox1* gene; phylogenetic analysis; Genetic diversity.

https://doi.org/10.36899/JAPS.2026.4.0101 Published first online May 21, 2026

INTRODUCTION

Earthworms, classified within the order Oligochaeta, play a pivotal role in improving and maintaining soil fertility by regulating its structure, ecological dynamics, and biogeochemical cycles (Thakuria *et al*., 2010; Groffman *et al*., 2015). As hermaphroditic organisms, they possess both male and female reproduction systems (Shen *et al*., 2012; De Sosa *et al*., 2017; Sun *et al*., 2017). They are distributed worldwide, particularly in tropical and subtropical regions, where they comprise 60-80% of soil macrofaunal biomass (Kim *et al*., 2022). Earthworms alter soil structure by degrading organic matter, improving nutrient cycling, and fostering beneficial microbes (Blouin *et al.,* 2013). Based on their activity and feeding habitats, earthworms are classified into Epigeic, Anecic, and Endogeic (Loza-Murguía *et al*., 2011). Approximately 300 species of the family Lumbricidae have been identified and recognized (Pérez-Losada *et al*., 2009). Various factors affect the diversity and distribution of earthworms, including parthenogeny, geological processes, climatic instability, and human activities (Novo *et al*., 2011; Dupont *et al*., 2015; Aspe and James, 2018).

However, traditional morphology-based taxonomy often fails to resolve cryptic Lumbricidae species, and similar morphologies are classified as a single species (Pérez-Losada *et al*., 2009). Although traditional identification of earthworms relies on morphology, it is only a characterization, and morphological identification poses difficulties because it does not distinguish among closely related species. Hence, molecular techniques as well as morphological taxonomy will be essential for more accurate and precise identification of earthworms (King *et al*., 2008). Recently, molecular tools such as the polymerase chain reaction (PCR) have been used to identify DNA markers (*Cox1* and 18S *rRNA* genes) and effectively assess genetic variation among closely related earthworm species (Thakuria *et al*., 2010).

Mitochondrial DNA is commonly used for genetic analysis among closely related species because mitochondrial DNA evolves more rapidly than nuclear DNA and has a higher mutation rate (Arbogast, 2001; Pop *et al*., 2003). The *Cox1* gene is one of the most commonly used mitochondrial DNA genes, encoding the enzyme cytochrome c oxidase, and is widely used for DNA barcoding to study and assess the genetic diversity of morphologically similar earthworm species (King *et al*., 2008). Several studies have used DNA barcoding of the *Cox1* gene to identify unknown species of spiders, springtails, scorpions, and other invertebrates (Liu *et al*., 2014; Ali *et al*., 2020; Ashraaf *et al*., 2020).

Biodiversity analysis of earthworms is vital from economic and agricultural perspectives. In Iraq, most studies focus on the morphological identification of local earthworm species. Therefore, our study represents the first attempt to estimate the genetic structure of *Aporrectodea* species and *Pithemera bicincta* using DNA phylogenetic relationships among species collected from different areas of Duhok province.

MATERIALS AND METHODS

Samples Collection and Study Area: The present study examined dissected earthworms collected from the Badini region (approximately 36.84°-37.25° N latitude and 42.68°-42.84° E longitude), which was selected for its strategic location along international transit routes linking the Kurdistan Region of Iraq to Turkey and Iran. In total, 200 earthworms were collected from garden soil at several locations: Batifa (37.20° N, 43.02° E), Derkar (37.20° N, 42.82° E), Akre (36.84° N, 43.03° E), Sheladze (37.02° N, 43.80° E), Hiror (37.25° N, 43.22° E), Semel (36.85° N, 42.84° E), Duhok (36.84° N, 42.99° E), Zakho (37.14° N, 42.68° E), Amedia (37.10° N, 43.5° E), Hizawah (37.17° N, 42.81° E) and Peshabir (36.90° N, 42.75° E). The collected Earthworms were washed with clean water to remove any excess dirt and soil. All collected earthworms were kept in separate plastic jars, labelled with the area from which they were collected, and transported to the animal physiology lab at the University of Zakho for further processing. In addition, 20 Lumbricidae sequences were downloaded from GenBank.

Amplification of the *Cox1* gene: Approximately 20 mg of grouped gizzard was collected from earthworms, and mitochondrial DNA was extracted using the AddPrep Genomic DNA Extraction Kit (Sigma-Aldrich, Germany) according to the manufacturer's instructions. Mitochondrial *Cox1* gene was used as genetic marker for molecular identification. Universal primer forwards (*Cox1F*) 5'GGTCAACAAATCAAAAGATATTGG'3 and Reverse primer (*Cox1R*) 5'GGGATTCGTTCTAGGCG'3 were used to amplify 523 bps (Huang *et al.,* 2007). Each sample was prepared in a mixture (40 µL) using 1× Master mix (20 µL) containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.25 U of Taq DNA polymerase, 10 µM of each *Cox1* -F primer and *Cox1* -R (4 µL) and 25 ng/µL of DNA template (4 µL), 8 µl Distilled water.

The polymerase chain reaction (PCR) of *Cox1* gene was carried out using setting as follows: Initial denaturation for five minutes at 95°C, followed by 25 cycles of denaturation for thirty seconds at 94ºC, annealing for forty-five seconds at 60ºC, and extension for ninety seconds at 72ºC then ten min at 72ºC (final extension) (Huang *et al.,* 2007). Then the PCR product was observed on a 2% agarose gel stained with Redsafe dye and examined under an ultraviolet illuminator.

Sanger sequencing and phylogenetic analysis: Twelve PCR products were bidirectionally sequenced by commercial service (Macrogen, South Korea). The DNA sequences were aligned and trimmed using BioEdit (7.2) software and were identified using BLAST. All newly identified variants were submitted to NCBI GenBank for registration. The phylogenetic tree for earthworms was constructed using MEGA software (version 12). Muscle methods were used for alignment of DNA sequences, and a Maximum-Likelihood Tree was constructed using the Tamura-Nei model to compare the obtained sequences with the available corresponding nucleotide sequences and previously published sequences.

Haplotype network and Genetic diversity indices: Sequences and their GenBank accession numbers used to construct the phylogenetic tree were also used to organize the haplotype network. Different indices of genetic diversity, such as haplotype and nucleotide diversity, were estimated using the DnaSP6 program (Rozas *et al*., 2017).

RESULTS

Genotyping of earthworms: The PCR amplification of all samples revealed a 523 bp segment of the *Cox1* gene, which matched sequences in the GenBank (Figure 1). Based on BLAST searches, seven isolates were identified as *Aporrectodea trapezoides*, three as *Aporrectodea longa*, and two as *Pithemera bicincta.* The isolates were taxonomically identified and recorded in GenBank under the following accession numbers: PX229726-PX229728 were identified as *A. longa* isolates. The isolates with accession numbers PX229723-PX229725, PX229729-PX229731, and PX229733 were

identified as *A. trapezoides.* Two isolates with accession numbers PX229732 and PX229734 were identified as *P. bicincta* (Table 1). Two new species were recorded for the first time in Iraq: (i) *P. bicincta* found on Hizawah and Zakho; (ii) *A. longa* found in Batifa, Hiror and Derkar.

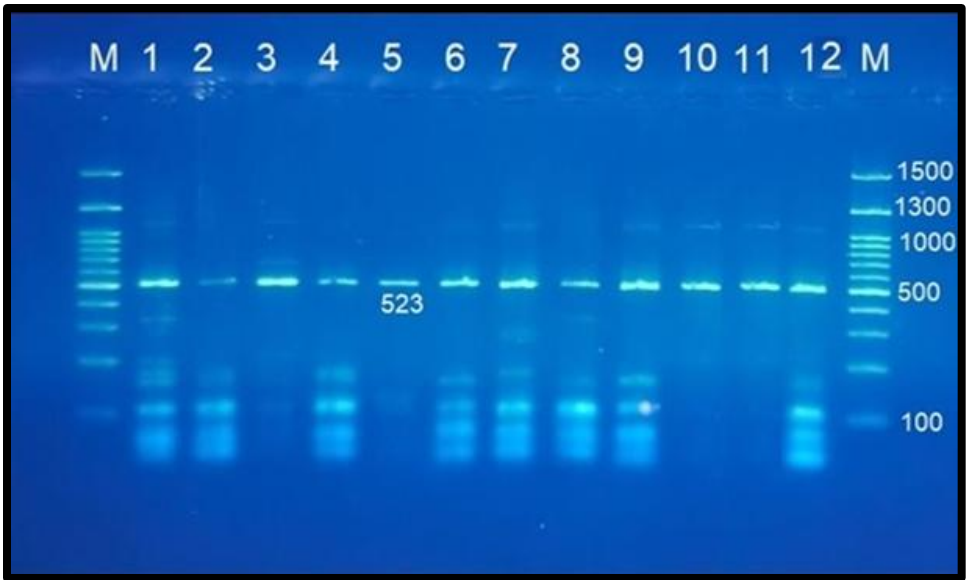

Figure 1: Agarose gel electrophoresis (2%) of Single-PCR products. Lane (L): DNA ladder (100-1500 bp). Lanes S1-S12 showed a 523 bp amplicon with *Cox1* gene primers.

Table 1: Accession numbers recorded in this study and related isolates

No.	*Species*	GenBank accession No.	Origin
1	*Aporrectodea trapezoids*	PX229723	Akre
2	*Aporrectodea trapezoids*	PX229724	Sheladze
3	*Aporrectodea trapezoids*	PX229725	Amedia
4	*Aporrectodea trapezoids*	PX229729	Peshabir
5	*Aporrectodea trapezoids*	PX229730	Duhok
6	*Aporrectodea trapezoids*	PX229731	Semel
7	*Aporrectodea trapezoids*	PX229733	Zakho
8	*Aporrectodea longa*	PX229726	Batifa
9	*Aporrectodea longa*	PX229727	Hiror
10	*Aporrectodea longa*	PX229728	Derkar
11	*Pithemera bicincta*	PX229732	Hizawah
12	*Pithemera bicincta*	PX229734	Zakho

Phylogenetic analysis: Maximum Likelihood (ML) analysis was performed based on *Cox1* nucleotide sequences. The phylogenetic trees resolved two main clades; the first clade contained two sequences (PX229726 and PX229728) clustered with *A. longa*, confirming their identity within this species. The phylogenetic tree (Fig. 2) resolved two main clades: the first clade contained two Iraqi sequences (PX229726, PX229728) that clustered with reference sequences of *A. longa*, confirming their identity as this species. Seven sequences (PX229723, PX229724, PX229731, PX229730, PX229725, PX229733, PX229729) grouped within the *A. trapezoides* clade, supported by bootstrap values ≥92%, which were closely related to reference sequences from Spain. Additionally, two Iraqi sequences (PX229732 and PX229734) clustered with the *P. bicincta* reference sequence from Japan with strong support (bootstrap 95%), confirming their identity as this species. These results highlight both species diversity among Iraqi earthworms and their phylogenetic relatedness to globally distributed lineages.

Haplotype network: Out of 32 sequences analyzed, only five haplotypes were identified (Hap_1-Hap_5) based on geographical location. The haplotype network based on *Cox1* gene revealed that the most prevalent haplotype was Hap_1 which present in the following isolates from Iraq (PX229726, PX229728, PX229723, PX229724, MW354675,

PX229724, PX229727, PX229731, PX229730, PX229725, PX229729 and PX229733); Spain (JF918620 and GU013952); China (KF205975 and EF077602); Iran (MT968742 and OR475033); USA (LC475695); Sweden (KT073946); Japan (AB543230); France (MT501890, OL979104 and PP166227); Canada (MG421571) and Portugal (JN850551). The remaining isolates, such as LT900527 from the UK, were found in Hap_2; MH882856 and MH882558 from China were found in Hap_4; MT502062 from France was found in Hap_5; and all isolates of *P. bicincta* from Iraq (PX229734 and PX229732) and Japan (AB596847) were found in Hap_3. Twenty-one-to-seventy-seven-point mutations were detected between haplotypes (Fig. 3).

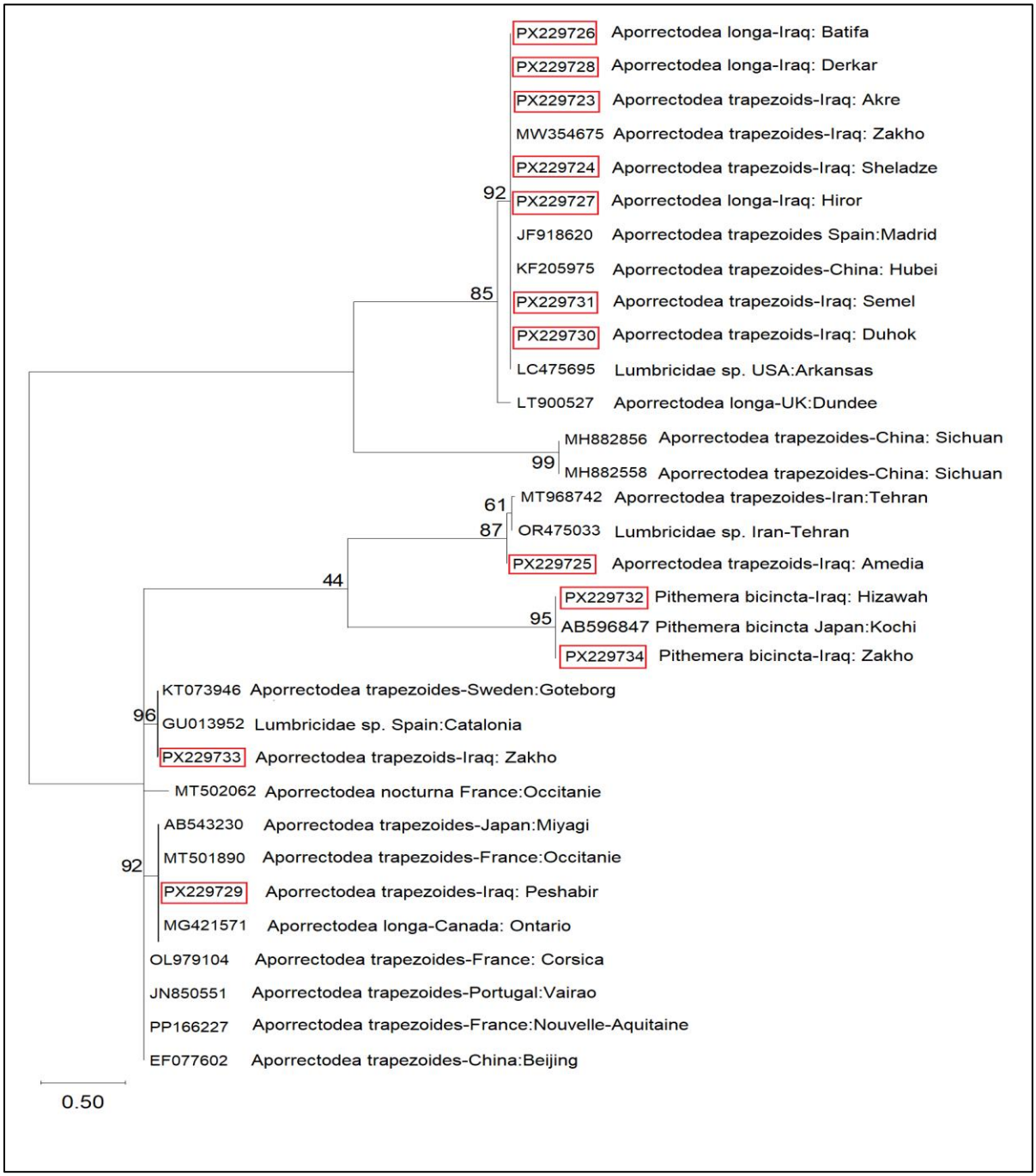

Figure 2: The phylogenetic Tree based on the *Cox1* gene in *A. trapezoides, A. longa* and *P. bicincta* from the Kurdistan region of Iraq using Maximum Likelihood methods.

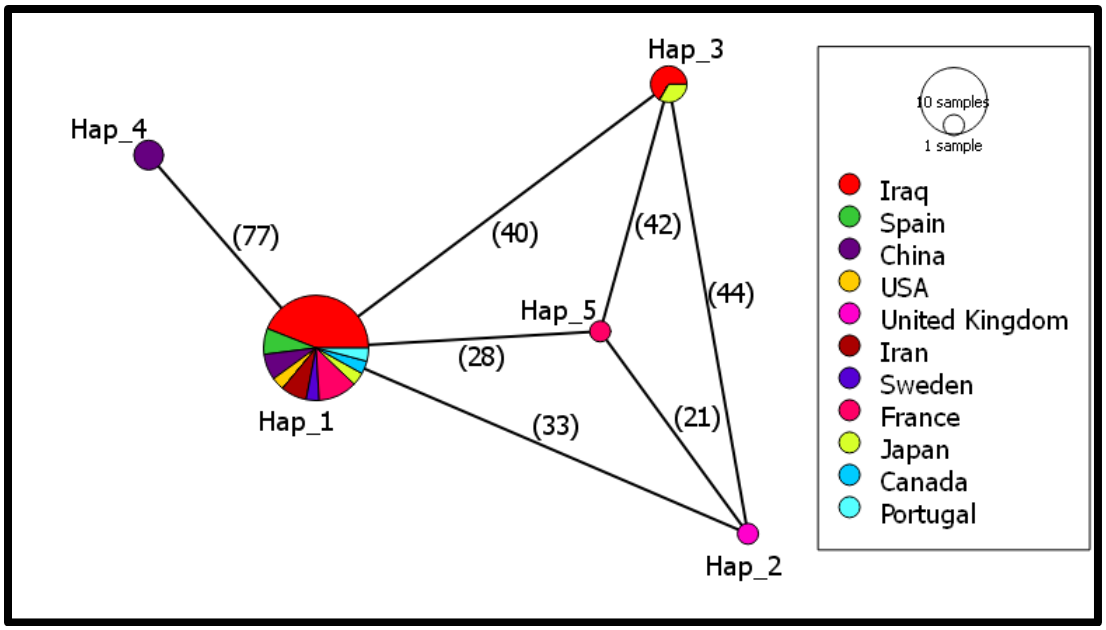

Fig. 3. Haplotype network of *A. trapezoides, A. longa* and *P. bicincta* based on the *Cox1* (523 bp) gene sequences. Five haplotypes were recorded (Hap 1- Hap 5). Different colors indicate the geographic distribution of haplotypes. The number in brackets represents the nucleotide change.

The international distribution map of the *Cox1* haplotypes (Fig. 4) indicated that Hap_1 was the most prevalent, and was found in Iraq, Spain, Iran, United Kingdom, France, Japan, Canada and Portugal. A result was that Hap 2 was confined to Europe, and Hap 3 had been detected in Iraq and Japan. Hap_4 was seen in China alone and Hap 5 in France alone. The highest percentage of samples to Hap_1 was contributed by Iraq which made it be the most predominant haplotype in the dataset. The occurrence of Hap_1 in several continents underscores its extensive spread, whereas the few remaining Haplotypes are localized, showing a limited occurrence of Haplotype variation.

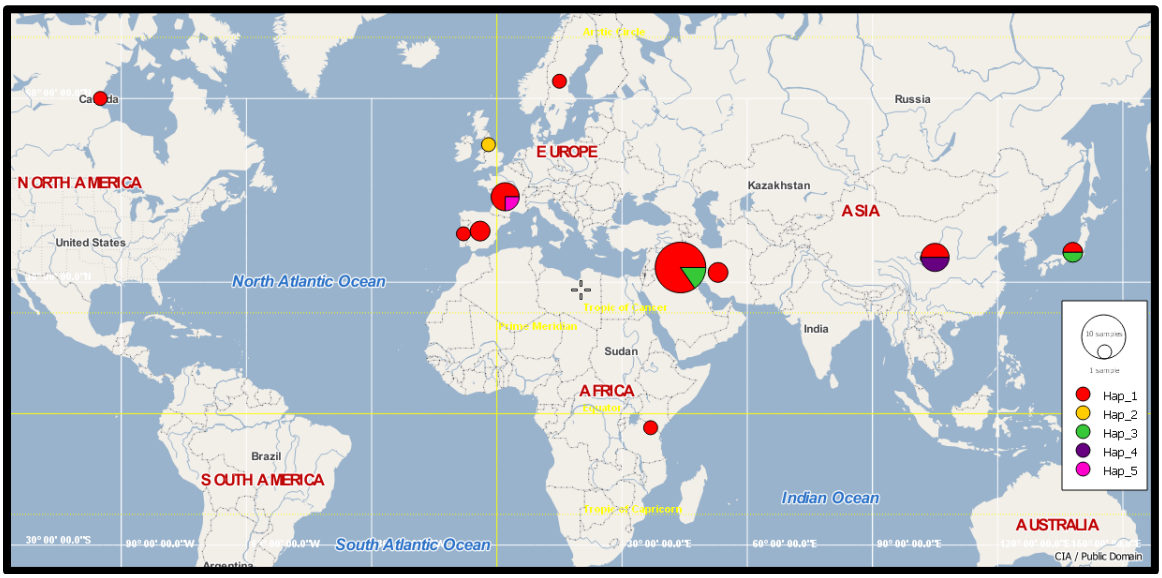

Figure 4: Haplotype distribution map of five haplotypes of isolates in the current study. The Haplotype map was built in PopART based on the mitochondrial *Cox1* sequences. The highest number of haplotypes (h =5) was in Iraq.

Nucleotide sequence variation, diversity and neutrality indices: Population genetic pointers were determined based on the nucleotide sequences of Iraqi and other countries in the *Cox1* genes. *Cox1* gene sequences of *A. trapezoides*, *A.*

longa and *P. bicincta* showed a moderate genetic variation between the analyzed isolates (n = 32). Five haplotypes were observed and the haplotype diversity (Hd) was 0.387, which is relatively low considering the haplotype variation in the population. The estimated nucleotide diversity (π) was 0.10189, which indicates a significant level of sequence divergence. The mean pairwise nucleotide difference (k) was 19.258 and 105 polymorphic sites with 133 mutations were identified by scanning through the sequences. The results of neutrality tests revealed that the Tajima D value (-1.58694) was negative, indicating that the low-frequency polymorphisms were too many, possibly because of population growth or purifying selection. Conversely, the FLD (1.38481) and FLF (0.44439) values of Fu and Li were positive, which reflects the effect of balancing selection or population structure, as it is in Table 2. Overall, the diversity and the neutrality indices reveal multifaceted evolutionary processes of the studied populations of earthworms.

Table 2: Diversity and neutrality indices based on *COX1* gene

Number of isolates (n)	32
Number of haplotypes (Hn)	5
Haplotype diversity (Hd)	0.387
Nucleotide diversity (π)	0.10189
Fu and Li's D* test statistic (FLD)	1.38481
Fu and Li's F* test statistic (FLF)	0.44439
Average number of pairwise nucleotide differences (k)	19.258
Tajima's D	-1.58694
Number of polymorphic (segregating) sites (S)	105
Total number of mutations	133

DISCUSSION

Both invasive and native earthworm species are revealed in the study. Since earthworms are economically and agriculturally important to the soil, the distribution and genetic diversity of earthworms have attracted the interest of international researchers, particularly in the Kurdistan region of Iraq, which is abundant in soils (Minamiya *et al*., 2009; Decaens *et al*., 2013). Some morphological identification of earthworms has been conducted, but few have been reported on the molecular characterization of earthworms, and their genetic diversity has been poorly known in Iraq (Othman and Ahmad, 2020; Aljbouri and Mahmood, 2023). Earthworms are hermaphroditic, which means that they possess both male and female reproductive organs. Lumbricus species reproduce sexually, and two adult worms' mate in which the sperm and eggs are exchanged during mating. This type of mating resulted in the emergence of variant haplotypes and higher genetic diversity (Shekhovtsov *et al*., 2024). Mitochondrial DNA barcoding has been extensively applied in investigating genetic variation because it has regions of conservation, moderate rate of evolution, and a fast rate of mutation (Kress and Erickson, 2008; Khan *et al.,* 2022). The knowledge of the genetics of earthworms is essential in giving information on how earthworms adapt to evolving environments and have a wider application on the ecology of the urban and conservation of the environment (Mautuit *et al*., 2024).

Our isolates of earthworms deposited in the GenBank database with accession numbers PX229726-PX229728 were the sequences of our isolates based on the *Cox1* gene. They were verified to be *A. longa* since they grouped with other related European sequences with high bootstrap. This observation indicates a global spread of lumbricids possessing a single evolutionary history. It reinforces the hypothesis that these species have been imported into Iraq by agricultural activities and soil erosion. On the other hand, other obtained sequences (PX229723, PX229724, PX229727, PX229729, PX229730, PX229731, and PX229733) were confirmed as *A. trapezoides*. Our results correlate with a study

conducted in Erbil, Iraq, which detected the earthworm species (including *A. trapezoides* and *A. longa*) in Duhok, Erbil, and Sulaymaniyah (Othman and Ahmad, 2020). In another study carried out in Baghdad, five species of *A. trapezoides* were found in the north and south of the city (Kamal *et al*., 2023).

The high similarity between our obtained sequences and reference sequences highlights the efficiency of molecular tools in confirming species limits and enhancing the documentation of Iraqi earthworm diversity (Aljbouri and Mahmood, 2023). Phylogenetic analysis based on the *Cox1* gene revealed strong bootstrap support between our sequences of *A. trapezoides* and Asian and European sequences, which suggests that the Iraqi isolates shared a recent evolutionary connection with globally dispersed sequences. *A. trapezoides* is ecologically flexible and tolerant of different soil types, and can survive in disturbed environments; hence, it explains its establishment in Iraq (Fernández, 2013). The presence of *A. trapezoides* and *A. longa* in Iraq supports the idea that soil and plant trade, as well as agricultural practices, may play a vital role in establishing these species in the region (Othman and Ahmad, 2020; Aljbouri and Mahmood, 2023).

A most significant finding was that identification of *P. bicincta* was recorded in the GenBank database under accession numbers (PX229732 and PX229734) and clustered with high bootstrap support with *P. bicincta* isolated from Japan. To the best of our knowledge, *P. bicincta* isolates were first reported in Iraq using DNA barcoding based on the *Cox1* gene. Such species are considered invasive and may have been introduced from China and Japan; their discovery in the Kurdistan region of Iraq suggests introduction via plant and soil transport. Such results will aid in understanding the distribution of *P. bicincta* in Iraq and its ecological impact. Overall, finding native species (*A. trapezoides* and *A. longa*) and the invasive species (*P. bicincta*) in northern Iraq may assist in understanding the richness of the Iraq ecological environment. Such species that are genetically dissimilar could promote soil structure, and nutrient cycling, thereby alleviating earthworm populations in Iraq (Othman and Ahmad, 2020; Aljbouri and Mahmood, 2023). Concerning the haplotype net, there were five haplotypes that were rearranged. The most prevalent and the global haplotype was haplotype 1. It was observed that Haplotypes 1 were prevalent in Iraq, Spain, China, Iran, the USA, Sweden, Japan, France, Canada, and Portugal indicating a common origin and well-adapted to new environmental needs (Dupont *et al*., 2015; Aspe and James, 2018; Klein *et al*., 2020). The dissemination of the five haplotypes signified extensive and regional trends of genetic variation (Torres-Leguizamon et al., 2014; Phillips *et al.,* 2019; Ganault *et al*., 2024; Mautuit *et al*., 2024). The neutrality and diversity index using the *Cox1* gene shows moderate haplotypic, and high levels of nucleotide variation in *A. trapezoides, A. longa* and *P. bicincta*.

The number of identified haplotypes was five out of 32 isolates making the diversity of haplotype relatively low (Hd = 0.387). This indicates that only a small number of dominant haplotypes are common and generally haplotype diversity is low. Conversely, the nucleotide diversity (0.10189) and the mean number of pairwise differences (19.258) are large, an indication of deep sequence divergence. The difference between low haplotype diversity and high nucleotide diversity suggests that the data are a limited set of highly divergent haplotypes, which is also consistent with the idea that several species have been included. The high polymorphic sites (S = 105) and overall mutations (133) also support the fact that there was much sequence variation on the nucleotide level.

Results of neutrality tests give opposite indicators. The result of Tajima's D was a negative (-1.58694), suggesting an excess of rare alleles could be due to the recent population explosion or they were undergoing some form of purifying pressure. Conversely, Fu and Li's D* (1.38481) and F* (0.44439) were positive indicating a relative deficit of singletons, perhaps due to population structure or balancing selection. This level of inconsistency between neutrality tests is presumably explained by the mixing of different species within a common dataset, which generates patterns that would not be predicted by the pattern of a single panmictic population (Sharma *et al*., 2011). In general, these data indicate that interspecific evolution, but not exclusively intraspecific mechanisms, contribute to the described genetic variation. Further analyses by evaluating diversity and neutrality indices of individual species would help in obtaining a more accurate yet realistic view of demographic history and selective forces.

Conclusion: The phylogenetic analysis confirms that earthworms gathered in northern Iraq are principally A. trapezoides and A. longa, with signs of genetic clustering with European and Asian groups. Also, the occurrence of P. bicincta in Iraq is a record native, enlarging the range of the species.

Conflict of interest: The authors declare no conflict of interest.

Funding: This study did not receive any funding support.

Authors' contributions: Nazik M. Othman, Sherwan T. Ahmad and Sofyan H. Sedo worked on the piece in equal measures. All writers were involved in study conception and design, data collection, data analysis and interpretation and manuscript preparation. All authors read and approved the final version of the manuscript.

REFERENCES

Ali, I., A. Khan, T.U. Rehman, M. Naseer, S.A. Khan and H. Khan (2020). Phylogenetic and evolutionary revision of some pheasants of northern Pakistan. Acta Entomol. Zool. 1(1):13-25. https://doi.org/10.33545/27080013.2020.v1.i1a.4.

Aljbouri, A.K. and S.M. Mahmood (2023). Evaluation the Effect of Hemazole Pesticide on the Vitality, Growth and Tissue Changes of Earthworms, Iraqi J. Agric. Sci., 54(3):667-673. https://doi.org/10.36103/ijas.v54i5.

Arbogast, B.S. (2001). Phylogeography: the history and formation of species. Am. Zool. 41(1):134-135. https://doi.org/10.1093/icb/41.1.134

Ashraaf, S., H.M. Tahir and S. Naseem (2020). Assessing the diversity of Cursorial spiders using DNA barcoding. Pak. J. Zool. 1-10. doi.org/10.17582/journal.pjz/20180117170115.

Aspe, N.M. and S.W. James (2018). Molecular phylogeny and biogeographic distribution of pheretimoid earthworms (clitellata: Megascolecidae) of the Philippine archipelago. Eur. J. Soil Biol. 85:89-97. https://doi.org/10.1016/j.ejsobi.2018.02.001.

Blouin, M., M.E. Hodson, E.A. Delgado, G. Baker, L. Brussaard, K.R. Butt, J. Dai, L. Dendooven, G. Peres, J.E. Tondoh, D. Cluzeau and J.J. Brun (2013), A review of earthworm impact on soil function and ecosystem services. Eur. J. Soil Sci. 64: 161-182. https://doi.org/10.1111/ejss.12025.

De Sosa, I., D.F. Marchán, M. Novo, D.J.D. Cosín, G. Giribet and R. Fernández (2017). Insights into the origin of parthenogenesis in oligochaetes: strong genetic structure in a cosmopolitan earthworm is not related to reproductive mode. Eur. J. Soil Biol. 81:31-38. https://doi.org/10.1016/j.ejsobi.2017.06.003.

Decaens, T., D. Porco, R. Rougerie, G.G. Brown and S.W. James (2013). Potential of DNA barcoding for earthworm research in taxonomy and ecology. Appl. Soil Ecol. 65:35-42. https://doi.org/10.1016/j.apsoil.2013.01.001.

Dupont, L., Y. Graville, B. Richard, T. Decans and J. Mathieu (2015). Dispersal constraints and fine-scale spatial genetic structure in two earthworm species. Biol. J. Linn. Soc. 114(2):335-347. https://doi.org/10.1111/bij.12436.

Fernández, R., A. Almodóvar, M. Novo, M. Gutiérrez and D.J.D. Cosín (2013). Earthworms, good indicators for palaeogeographical studies? Testing the genetic structure and demographic history in the peregrine earthworm *Aporrectodea trapezoides* (Dugès, 1828) in southern Europe. Soil Biol. Biochem. 58:127-135. https://doi.org/10.1016/j.soilbio.2012.10.021.

Ganault, P., C. Ristok, H.R.P. Phillips, M. Hedde, Y. Capowiez, N. Bottinelli and N. Eisenhauer (2024). Soil BON Earthworm-A global initiative on earthworm distribution, traits and spatiotemporal diversity patterns. Soil Org. 96(1): 47-60. https://doi:10.25674/362.

Groffman, P.M., T.J. Fahey, M.C. Fisk, J.B. Yavitt, R.E. Sherman, P.J. Bohlen and J.C. Maerz (2015). Earthworms increase soil microbial biomass carrying capacity and nitrogen retention in northern hardwood forests. Soil Biol. Biochem. 87:51-58. https://doi.org/10.1016/j.soilbio.2015.03.025.

Huang, J., Q. Xu, Z.J. Sun, G.L. Tang and Z.Y. Su (2007). Identifying earthworms through DNA barcodes. Pedobiologia. 51(4): 301-309. https://doi.org/10.1016/j.pedobi.2007.05.003

Kamal, S.B., F. Chachain Nebrass and A. Sabbah Majeed (2023). Morphological and Molecular Identification of *Aporrectodea trapezoides* (Duges, 1828) in Baghdad. Res. J. Biotechnol. 18(5): 78-82. https://doi.org/10.25303/1805rjbt078082

Khan, M.U., S. Andleeb, M.F. Khan and R.G. Mustafa (2022). Molecular characterization and phylogenetic analysis of earthworm species collected from different soil habitats of Poonch division Azad Jammu and Kashmir Pakistan. J. Oleo Sci. 71(9): 1349-1361. https://doi.org/10.5650/jos.ess21450.

Kim, G., H. Jo, H.S. Kim, M. Kwon and Y. Son (2022). Earthworm effects on soil biogeochemistry in temperate forests focusing on stable isotope tracing: a review. Appl. Biol. Chem. 65, 88. https://doi.org/10.1186/s13765-022-00758-y.

King, R.A., A.L. Tibble and W.O. Symondson (2008). Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. Mol. Ecol. 17(21): 4684-4698. https://doi.org/10.1111/j.1365-294X.2008.03931.x

Klein, A., N. Eisenhauer and I. Schaefer (2020). Invasive lumbricid earthworms in North America—Different life histories but common dispersal. J. Biogeogr. 47(3): 674-685. https://doi.org/10.1111/jbi.13744.

Kress, W.J. and D.L. Erickson (2008). DNA barcodes:genes, genomics, and bioinformatics. Proc. Natl. Acad. Sci. 105(8): 2761–2762. https://doi.org/10.1073/pnas.0800476105.

Liu, X.F., C.H. Yang, H.L. Han, R.D. Ward and A.B. Zhang (2014). Identifying species of moths (Lepidoptera) from Baihua Mountain, Beijing, China, using DNA barcodes. Ecol. Evol. 4(12): 2472-2487. https://doi.org/10.1002/ece3.1110.

Loza-Murguía, M.G., R. Acho, A. Arce, R. Huaycho, N. Aruhiza and S. Mamani (2011). The population of earthworms Lumbricus spp. on the plateau of La Paz, Bolivia. Cienc. Investig. Agrar. 38(3): 415-423. http://dx.doi.org/10.4067/S0718-16202011000300011.

Mautuit, A., D.F. Marchán, S. Barantal, M. Brand, A. Lucas, J. Cortet and T. Decaëns (2024). Environmental drivers of genetic diversity and phylogeographic pattern in urban earthworms. Eur. J. Soil Biol. 121: 103620. https://doi.org/10.1016/j.ejsobi.2024.103620.

Minamiya, Y., J. Yokoyama and T. Fukuda (2009). A phylogeographic study of the Japanese earthworm, Metaphire sieboldi (Horst, 1883) (Oligochaeta: Megascolecidae): Inferences from mitochondrial DNA sequences. Eur. J. Soil Biol. 45(5-6):423-430. https://doi.org/10.1016/j.ejsobi.2009.06.004.

Novo, M., A. Almodóvar, R. Fernández, G. Giribet and D.J.D. Cosín (2011). Understanding the biogeography of a group of earthworms in the Mediterranean basin—The phylogenetic puzzle of Hormogastridae (Clitellata: Oligochaeta). Mol. Phylogenet. Evol. 61(1): 125-135. https://doi.org/10.1016/j.ympev.2011.05.018.

Othman, H.H. and S.T. Ahmad (2020). Molecular identification of some earthworm species (Annelida; Oligochaeta) in Kurdistan-Iraq. Zanco J. Pure Appl. Sci. 32(6): 46-53. https://doi.org/10.21271/ZJPAS.32.6.5.

Pérez-Losada, M., M. Ricoy, J.C. Marshall and J. Domínguez (2009). Phylogenetic assessment of the earthworm *Aporrectodea* caliginosa species complex (Oligochaeta: Lumbricidae) based on mitochondrial and nuclear DNA sequences. Mol. Phylogenet. Evol. 52(2): 293-302. https://doi.org/10.1016/j.ympev.2009.04.003.

Phillips, H.R., C.A. Guerra, M.L. Bartz, M.J. Briones, G. Brown, T.W. Crowther and N. Eisenhauer (2019). Global distribution of earthworm diversity. Science. 366(6464): 480-485. https://doi.org/10.1126/science.aax4851.

Pop, A.A., M. Wink, and V.V. Pop (2003). Use of 18S, 16S rDNA and cytochrome c oxidase sequences in earthworm taxonomy (Oligochaeta, Lumbricidae): The 7th international symposium on earthworm ecology· Cardiff· Wales· 2002. Pedobiologia. 47(5-6): 428-433. https://doi.org/10.1078/0031-4056-00208.

Rozas, J., A. Ferrer-Mata, J.C. Sánchez-DelBarrio, S. Guirao-Rico, P. Librado, S.E. Ramos-Onsins and A. Sánchez-Gracia (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34(12): 3299-3302. https://doi.org/10.1093/molbev/msx248.

Sharma, A., H. Sonah, R.K. Deshmukh, N.K. Gupta, N.K. Singh and T.R. Sharma (2011). Analysis of genetic diversity in earthworms using DNA markers. Zool. Sci. 28(1): 25-31. https://doi.org/10.2108/zsj.28.25.

Shekhovtsov, S.V., Y. Derzhinsky and E.V. Golovanova (2024). Earthworm (Oligochaeta, Lumbricidae) intraspecific genetic variation and polyploidy. Vavilov J. Genet. Breed. 28(5): 563–570. doi: 10.18699/vjgb-24-62.

Shen, H.P., H.T. Yu and J.H. Chen (2012). Parthenogenesis in two Taiwanese mountain earthworms Amynthas catenus Tsai *et al*., 2001 and Amynthas hohuanmontis Tsai et al., 2002 (Oligochaeta, Megascolecidae) revealed by AFLP. Eur. J. Soil Biol. 51:30-36. https://doi.org/10.1016/j.ejsobi.2012.03.007.

Sun, J., S.W. James, J. Jiang, B. Yao, L. Zhang, M. Liu and F. Hu (2017). Phylogenetic evaluation of Amynthas earthworms from South China reveals the initial ancestral state of spermathecae. Mol. Phylogenet. Evol. 115: 106-114. https://doi.org/10.1016/j.ympev.2017.07.026.

Thakuria, D., O. Schmidt, D. Finan, D. Egan and F.M. Doohan (2010). Gut wall bacteria of earthworms: a natural selection process. The ISME journal. 4(3):357-366. https://doi.org/10.1038/ismej.2009.124.

Torres-Leguizamon, M., J. Mathieu, T. Decaens and L. Dupont (2014). Genetic structure of earthworm populations at a regional scale: inferences from mitochondrial and microsatellite molecular markers in *Aporrectodea* icterica (Savigny 1826). PLoS One. 9(7): e101597. https://doi.org/10.1371/journal.pone.