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RESEARCH PAPER

Description and molecular characterization of *Longidorus orientalis* (Loof, 1982) associated with date palm (*Phoenix dactylifera* L.) in Iraq

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ABSTRACT

Soil samples from the rhizosphere of date palm (*Phoenix dactylifera* L.) trees were collected from orchards in Basrah Province, southern Iraq, a major region for date palm cultivation. This study aimed to isolate and identify plant-parasitic nematodes associated with date palms. Samples were collected from the root zone surrounding the trees, and nematodes were extracted using a sieving technique. Morphometric measurements were obtained from nematodes mounted on permanent slides. To confirm species identification, DNA was extracted from individual females, and the D2–D3 region of the 28S rRNA gene was amplified using PCR. Morphometric and molecular analyses confirmed the species *Longidorus orientalis*. Sequencing of the D2–D3 region (553 bp) showed 100% similarity with an Iranian isolate (GenBank accession no. GQ988722.1). The nucleotide sequence of the Iraqi isolate was deposited in GenBank under accession no. PV416847.1. This study represents the first record of L. orientalis associated with date palms in Iraq, highlighting its potential as a pathogenic threat and emphasizing the need for sustainable management strategies to reduce its impact and prevent further spread.

Keywords: D2-D3 region, *Longidorus orientalis*, molecular identification, morphometric analysis, rhizosphere

INTRODUCTION

Longidorus spp. are considered among the most significant plant-pathogenic nematodes belonging to the family Longidoridae, phylum Nematoda. These nematodes are morphologically characterized by elongated, slender, and filamentous bodies. They are soil-dwelling ectoparasites that feed externally on plant roots using a specialized stylet (odontostyle) measuring between 80 and 260 µm in length (Archidona-Yuste et al., 2016; Kantor et al., 2024). *Longidorus* spp. represent the second most significant and diverse genus in the family Longidoridae, following *Xiphinema*, with more than 180 described species (Kantor et al., 2024). These pathogenic nematodes have a worldwide distribution, particularly in temperate regions, and exhibit high infectivity toward a wide range of host plants, including fruit trees, herbaceous plants, vegetables, and other crops. This broad distribution of *Longidorus* nematodes highlights their significant economic impact on the agricultural sector (Peneva et al., 2001, Archidona-Yuste et al., 2016).

Damage to infected plants caused by these nematodes is associated with their feeding behavior on the root system, resulting in root deformation and the appearance of small swellings or galls, as well as impaired water and nutrient uptake. These physiological disruptions lead to a significant reduction in plant growth and productivity (Cai et al., 2020). In addition, certain *Longidorus* species are well known for transmitting plant-pathogenic viruses, particularly nepoviruses, which cause severe diseases and substantial crop losses (Archidona-Yuste et al., 2016; Kantor et al., 2024).

Accurate identification of *Longidorus* spp. remains challenging due to the high degree of similarity in their morphological features (Mobasseri et al., 2023). This complexity necessitates the use of integrative taxonomic approaches that combine traditional morphological methods with modern molecular techniques. In particular, DNA sequencing of genetic markers such as the D2–D3 expansion segments of the 28S rDNA has proven to be a reliable and effective method for the identification and characterization of *Longidorus* spp., as well as for the description of new taxa (Ehtesham et al., 2023; Kornobis, 2023).

Recently, several studies have reported the discovery of new *Longidorus* species in various countries worldwide, including Iran, Spain, and the United States. These findings enhance our understanding of the diversity and geographical

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distribution of the genus *Longidorus* (Mobasseri et al., 2023; Clavero-Camacho et al., 2021; Kantor et al., 2024). Management of *Longidorus* species requires comprehensive strategies, including the adoption of good agricultural practices, the use of resistant plant varieties, and the application of biological control agents (Archidona-Yuste et al., 2016).

The present study aimed to identify plant-parasitic nematodes from the rhizosphere of date palms in Basrah Province, Iraq, using morphometric assessment and molecular analysis targeting the D2–D3 region of the nematode 28S rDNA.

MATERIALS AND METHODS

Sample Collection, Extraction, and Morphological Identification. Soil samples representing the rhizosphere of *Phoenix dactylifera* L. trees were collected from orchards located in Basrah Governorate, southern Iraq, one of the primary regions for date palm cultivation. Samples were collected from a soil depth ranging from 20 to 40 cm. Approximately 1 kg of soil was collected per sample. The samples were placed in sterile plastic bags and transported to the laboratory under refrigerated conditions to preserve nematode viability.

Nematodes were extracted from the soil samples using the sieving technique, which involves passing a soil-water mixture through a series of sieves with decreasing mesh sizes to separate nematodes from larger soil particles (Brown & Boag, 1988).

Two hundred grams of soil were mixed with an appropriate volume of distilled water in a plastic container and stirred thoroughly to release nematodes from the soil matrix. The mixture was then sequentially filtered through a coarse sieve (2 mm), a finer sieve (100 µm), and finally a fine sieve (38 µm) to obtain a concentrated nematode suspension.

Following extraction, nematodes were collected from the water retained beneath the sieves using small tubes. The nematodes were subsequently heat-killed and fixed in glycerin following the method of De Grisse (1969). Morphometric measurements were conducted using a Leica DM300 microscope equipped with a camera lucida. Photomicrographs were captured using an Opto-Edu (China) microscope connected to a digital camera.

DNA Extraction, Polymerase Chain Reaction (PCR), and Sequencing. Single female nematodes were selected in a drop of distilled water (H_2O) on a temporary slide under a light microscope. Each specimen was transferred to 5 µL of TE buffer (10 mM Tris-Cl and 0.5 mM EDTA, pH 9.0) placed on a clean slide, and crushed using a coverslip. Subsequently, 15 µL of the same buffer was added to collect the sample, and the DNA extracts were stored at –20 ºC until used as templates for polymerase chain reaction (PCR).

Specific primers were used to amplify regions of ribosomal DNA (rDNA). For amplification of the LSU rDNA D2–D3 expansion segments, the forward primer D2A (5'-ACA AGT ACC GTG AGG GAA AGT-3') and the reverse primer D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') were used, following Nunn (1992). PCR reactions were performed under the the conditions described by Archidona-Yuste et al. (2016) in a total reaction volume of 50 µL using Pfu DNA polymerase (Korea).

Phylogenetic Analyses. The PCR amplicons (~553 bp) were purified and sequenced by Macrogen Inc. (Korea). The obtained sequences were subjected to BLAST searches using the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). All sequences corresponding to the D2-D3 expansion segments of the 28S rRNA gene were aligned using ClustalW implemented in MEGA version 11. A phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replicates to assess branch support.

RESULTS AND DISCUSSION

Morphological Characterization of Longidorus orientalis. The extracted nematode possesses a cylindrical body that tapers towards the anterior region. Upon death, the body curves ventrally to form a C-shape, and in some specimens, it coils helically. Females are characterized by a rounded, continuous lip region with bilobed amphidial pouches and a hemispherical tail. The cuticle is smooth, with a thickness ranging from 2.5 to 3 µm at the mid-body. The guiding ring is single and measures 4–5 µm in width. The odontostyle is long, slender, needle-like, straight or slightly curved, with a slightly broader basal attachment. The esophagus is typical for this species, with a cylindrical basal bulb. The female reproductive system is didelphic, consisting of two equally developed branches, anterior and posterior. The vulva is slightly anterior to the mid-body, appearing as a transverse slit measuring 12–14 µm in width. The vagina is strongly sclerotized and extends inward to about half the body radius (Figure 1).

Morphometric Comparison with Reference Populations. Morphological variation was observed in several traits between the Iraqi population and reference population of *L. orientalis,* as shown in Table 1 and Figure 1. The Iraqi population exhibited a body length ranging from 3856.0 to 3972.0 µm, which was close to that of the standard isolate (3805–4554 µm). The "a" ratio ranged from 68.0 to 71.1, which was lower than that of the comparable isolate (82.5–106). The "b" ratio varied from 8.8 to 9.5, also lower than that of the standard isolate (11.0–13.7). The "c" ratio ranged from 145.9 to 153.7, which was higher than that of the comparable isolate (104–134). The "V" ratio ranged from 51.0 to 53.6, which is approximately similar to

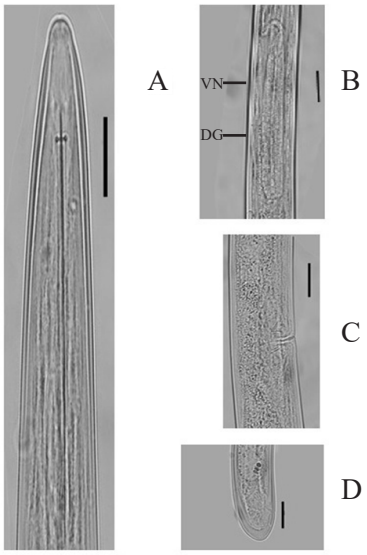

Figure 1. Light micrographs of *Longidorus orientalis*. A. Female anterior body region showing the odontostyle, odontophore, and guiding ring; B. Detail of the basal bulb showing the dorsal gland and ventrosublateral nuclei (arrowed); C. Vulval region; D. Female tail. Scale bars = 20 µm.

Table 1. Morphometric characteristics of *Longidorus orientalis* from Iraq

Character	Paratype females	Holotype female (Subbotin et al., 2015)
n	10.0	10.0
L	3911.3 ± 44.7 (3856.0–3972.0)*	4236 ± 261 (3805–4554)
L'	3885.1 ± 44.4 (3829.6–3945.7)	_____
a	69.5 ± 0.9 (68.0–71.1)	94.6 ± 7.5 (82.5–106)
b	9.2 ± 0.2 (8.8–9.5)	12.0 ± 0.7 (11.0–13.7)
c	149.4 ± 2.6 (145.9–153.7)	121.0 ± 9.4 (104–134)
V	52.5 ± 0.0 (51.0–53.6)	50 ± 2.42 (46–54)
V'	52.9 ± 0.0 (51.4–54.0)	_____
Odontostyle length	103.0 ± 0.0 (102.7–103.8)	91 ± 3.7 (88–97)
Odontophore length	85.8 ± 1.0 (83.8–86.7)	57 ± 3.9 (53–62)
Oesophagus	424.8 ± 7.7 (415.0–438.0)	354 ± 29.9 (315–410)
Head-Vulva	2055.1 ± 20.9 (2010.0–2082.0)	_____
Body Width(BW)	56.3 ± 0.8 (54.6–56.9)	_____
Anal Bady Width	38.9 ± 0.4 (25.6–26.6)	_____
Tail/ABW	26.2 ± 0.4 (25.6–26.6)	35 ± 1.3 (34–38)
Vul. / end	1856.2 ± 53.5 (1795.0–1934.0)	_____
Guiding ring from head	32.7 ± 0.5 (31.6–33.3)	_____

* Measurements are given in µm and expressed as mean ± SD (range).

that of the standard isolate (46–54). The odontostyle length ranged from 102.7 to 103.8 µm, exceeding that of the standard isolate (88–97 µm). The odontophore length ranged from 83.8 to 86.7 µm, which is greater than that of the standard isolate (53–62 µm). The esophagus length ranged from 415.0 to 438.0 µm, which is higher than the standard isolate measurement (315–410 µm).

Molecular Identification Based on 28S rRNA (D2–D3 Region). Molecular diagnosis of *Longidorus* sp. using the D2A and D3B primers targeting the D2-D3 expansion domains of the 28S rRNA gene confirmed species identity. PCR products (553 bp) were sequenced and analysed. BLAST results showed 100% sequence similarity between the Iraqi isolate and the Iranian *L. orientalis* isolate deposited under accession number GQ988722.1. The Iraqi isolate was deposited in GenBank under accession number PV416847.1.

Phylogenetic Analysis of *Longidorus orientalis*. Phylogenetic analysis highlighted the unique genetic position of the Iraqi isolate of *L. orientalis* (PV416847.1), which is clearly distinct from all other analyzed isolates. Positioned on a separate branch with the longest genetic distance (branch length = 0.0063), the Iraqi isolate exhibits the highest level of divergence within the group. Although it shares a common node with an Iranian isolate (GQ988722.1), as illustrated in the phylogenetic tree (Figure 2), the Iraqi isolate (PV416847.1; highlighted with a red border) remains genetically distant, suggesting potential geographic isolation or an independent evolutionary history (Baldwin et al., 2004). Bootstrap values indicate the robustness of the inferred relationships, and this analysis underscores the unique genetic status of the Iraqi isolate within the species complex (Table 2).

The efficiency of the D2-D3 expansion segments of the 28S rRNA gene in nematode diagnosis has been

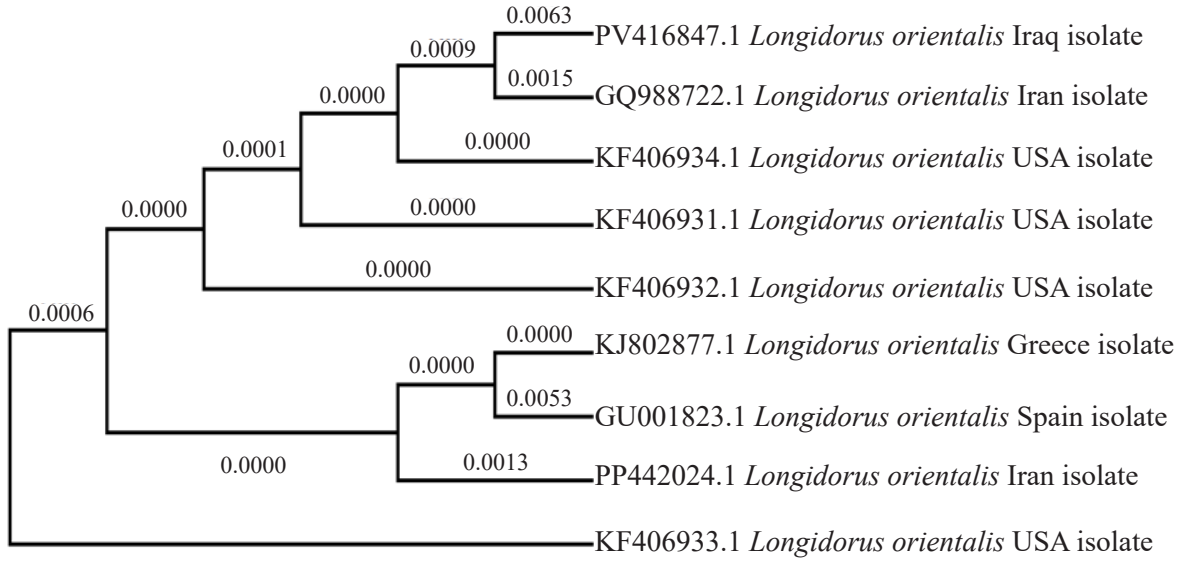

Figure 2. Phylogenetic relationship of *Longidorus orientalis* isolates from Basrah (PV416847.1) with related taxa using the Neighbor-Joining method based on the D2–D3 expansion domains of the 28S rRNA gene.

Table 2. Summary of BLAST-NCBI identity results for *Longidorus orientalis* isolates from Basrah using D2A and D3B primers

Country	Query cover %	Percentage identity %	Accession No.	Iraqi isolate *Longidorus orientalis*
Iran	100	99.458	GQ988722.1	PV416847.1
USA	100	99.458	KP406934.1	PV416847.1
USA	100	99.458	KP406932.1	PV416847.1
USA	100	99.458	KP406931.1	PV416847.1
Greece	100	99.458	KJ802877.1	PV416847.1
Iran	100	99.277	PP442024.1	PV416847.1
USA	100	99.096	KP406933.1	PV416847.1
Spain	100	98.741	GU001823.1	PV416847.1

well documented. Several studies have demonstrated the suitability of D2A and D3B primers for molecular studies of nematodes, including *L. orientalis* (Gutiérrez-Gutiérrez et al., 2013; Cai et al., 2020; Clavero-Camacho et al., 2021; Inserra et al., 2020). The present results are in a good agreement with the findings of He et al. (2005); Palomares-Rius et al. (2010); Archidona-Yuste et al. (2019), and Tzortzakakis et al. (2021) regarding *L. orientalis* identification.

CONCLUSION

The present study provides the first confirmed report of the plant-parasitic nematode *L. orientalis* associated with date palm (*P. dactylifera* L.) in Iraq, specifically from date palm orchards in Basrah Province. Species identification was supported by both morphometric characteristics and molecular analysis of the D2-D3 region of the 28S rRNA gene, which showed 100% sequence similarity with an Iranian isolate (GenBank accession no. GQ988722.1). The Iraqi isolate was deposited in GenBank under accession number PV416847.1, contributing to the growing database of nematode biodiversity in the region. The detection of *L. orientalis* in date palm rhizospheres highlights a potentially significant phytosanitary threat, particularly in a region that plays a major role in the country's date palm production. This finding underscores the urgent need for further surveys to determine the distribution and population dynamics of this nematode, as well as the implementation of sustainable management strategies to mitigate its potential impact on date palm cultivation in Iraq.

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AUTHORS' CONTRIBUTIONS

AZA proposed the study, collected specimens, and prepared the manuscript. OGB proposed the study, collected specimens, and prepared the manuscript. WAM proposed the study and conducted the genetic experiment. AHM proposed the current study, performed the bioinformatics analysis, and prepared the manuscript. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that they have no conflict of interests.

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