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# Morphometric and genetic variability among Mediterranean cereal cyst nematode (*Heterodera latipons*) populations in Turkey

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**Abstract:** The Mediterranean cereal cyst nematode, *Heterodera latipons*, is an important plant parasitic nematode that negatively impacts cereal production worldwide, particularly in the Mediterranean Basin. Successful control of cereal cyst nematodes requires knowledge of the nematode species/pathotypes and their biology. However, little information is currently available concerning the phenotypic and genotypic variability of *H. latipons* populations in Turkey. This study analyzed the morphological/morphometric and genetic characteristics of Turkish *H. latipons* populations to define intraspecific distinctions between them. Morphological and morphometric studies focused on the pattern of the perineal region of cysts and second stage juveniles (J2), while the molecular analyses used sequenced ITS-rDNA regions targeting the internal transcribed spacer (ITS) region of rDNA. The data from these investigations demonstrate that nematode populations from Adana and Hatay provinces differ from the populations collected in Kilis, Gaziantep, and Mardin provinces. These phenotypic and genotypic differences between *H. latipons* populations may indicate that they are heterogenic, with at least two pathotypes. To date, this study is the most comprehensive analysis identifying *H. latipons* populations in major wheat-producing areas of Turkey.

**Key words:** Cereals, *Heterodera latipons*, heterogeneity, ITS region rDNA, morphometrics, nematodes

## 1. Introduction

Plant parasitic nematodes are responsible for annual economic losses of up to US$ 100 billion worldwide (Urwin et al., 1997; Bird and Kaloshian, 2003). Cereal nematode genera such as *Heterodera* (cyst), *Meloidogyne* (root-knot), *Tylenchorhynchus* and *Merlinius* (stunt), *Ditylenchus* (stem), *Anguina* (seed-gall), *Pratylenchus* (root-lesion), and *Paratrichodorus* (stubby root) can cause significant yield losses (Rivoal and Cook, 1993; McDonald and Nicol, 2005). Among the cereal nematodes, cereal cyst nematodes (CCNs) are the most widely studied on wheat and have been reported by many researchers worldwide (Cook and Noel, 2002; Handoo, 2002; Nicol et al., 2003; Subbotin et al., 2003; Dababat et al., 2014, 2015).

*Heterodera* spp. are highly heterogeneous in their virulence on specific wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and oat (*Avena sativa*) genotypes (Cook and Noel, 2002; McDonald and Nicol, 2005; Dababat et al., 2015). CCN management strategies depend on knowledge of nematode species/pathotypes and their biological effects on cereals, as well as on the economically acceptable control options by farmers (Holgado et al., 2004). Identifying specific CCN species is therefore an essential component of selecting appropriate control strategies (Hyman, 1996). To date, researchers have identified 12 species and intraspecific CCN pathotypes infecting cereals and grasses; among them *H. filipjevi*, *H. latipons*, and *H. avenae* are the most damaging species (Rivoal and Cook, 1993; McDonald and Nicol, 2005). *H. latipons*, for example, has been reported on cereal crops such as wheat, barley, and oat throughout the Mediterranean region including Cyprus, Italy, Jordan, Israel, Spain, Morocco, Algeria, and Turkey (Franklin, 1969; Cohn and Ausher, 1973; Mor et al., 1992; Yousef and Jacob, 1994; Philis, 1995; Rumpenhorst et al., 1996; İmren et al., 2012; Haddadi and Mokabli, 2015; Mokrini et al., 2017).

Accurately identifying plant parasitic nematodes requires both morphological and molecular tools.

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Taxonomists previously identified nematodes to the species level using quantitative and qualitative characteristics, with heavy reliance on gross morphological and morphometric characterizations. The perineal region patterns of cysts and second stage juveniles (J2) are the most common features used to differentiate species of *Heterodera* (Handoo, 2002; Subbotin et al., 2003). Morphological and morphometric identification has been widely adopted, but morphological differentiation of *Heterodera* species is quite difficult and requires specialist skills. However, new molecular diagnostic techniques are overcoming the taxonomic limitations associated with conventional species identification (Szalanski et al., 1997; Al-Banna et al., 2004). For example, the internal transcribed spacer region (ITS) is variable and therefore useful for *H. avenae* group identification and phylogenetic studies at the species level (Subbotin et al., 2003).

It is important to understand the genetic variation within and/or between populations to develop resistant host plant and gene flow information. No research to date has comprehensively studied the phenotypic and genetic differences of *H. latipons* in Turkey. This study therefore aimed to compare morphological and morphometric characteristics of Turkish *H. latipons* populations with those previously reported, and to evaluate their genetic polymorphism based on ITS-rDNA sequences. It is difficult to reliably identify *H. latipons* using conventional methods because they occur with other *Heterodera* species in the same field. This study utilized morphological, morphometric, and molecular identification to distinguish individual *H. latipons* populations.

## 2. Materials and methods

### 2.1. *Heterodera latipons* populations

A total of 42 *Heterodera* populations were collected from wheat and barley fields in Turkey during the 2016 growing season. Of these, 26 populations were collected from the southeastern area (Gaziantep, Kilis, and Mardin provinces) and 16 populations from the eastern Mediterranean region (Adana and Hatay provinces), as shown in Table 1.

The wheat and barley fields surveyed displayed stunted patches, poor plant growth, chlorotic lower leaves, and few or no tillers. Cyst populations were collected from 42 different fields arbitrarily with separation distance of 7–10 km. A representative sample of 2 kg of soil consisting of 5–7 subsamples was prepared. A modified sieving-decanting method (Fenwick, 1940) was used to extract cysts from the soil. Cysts were identified to genus level under a stereo-binocular microscope (V20, Zeiss, Jena, Germany). At least ten cysts were collected for each population and stored at 4 °C for use in molecular and morphological analysis.

### 2.2. Morphological and morphometric identification

Each *H. latipons* population was identified based on the structures of the cysts' vulval cones, and J2 measurements and morphometric features. Vulval cone slides were prepared by fixing eight cysts each population in formalin-glycerol fixative mounted on glycerol and detected with a light microscope, according to Hooper (1986). We measured the length of the vulval slit, width of the vulval bridge, width of the fenestra, length of the fenestra, and length and width of the underbridge. The presence or absence of underbridge and bullae analysis of cyst perineal pattern were examined (Handoo, 2002).

Ten juveniles obtained from the same cysts were gently heated, fixed in triethanolamine formalin solution, embedded in glycerol, and then prepared on permanent slides (Handoo, 2002). We measured body length, stylet length, distance from anterior region to junction with the esophagus, body width, distance from anterior region to the base of the esophageal bulb, tail length, tail width, and length of the hyaline portion of the tail. The most important characters of J2 identification, a, b', c, and c' ratios and the ratio of hyaline portion to stylet length, were also calculated (Handoo, 2002). CCN populations were identified using previously established descriptions and diagnostic keys for cyst and J2 morphological characteristics (Franklin, 1969; Mulvey and Golden, 1983; Handoo, 2002). Ten J2s and one cyst for each population were observed, photographed, and measured using a Leica DFC295 digital camera installed on a Leica DM5000 B optical microscope and Leica Application Suite (LAS) software v.4.1.0.

Data were analyzed using analysis of variance (ANOVA) procedures in SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA) to determine any noteworthy differentiations among the 42 populations ($P \leq 0.05$). A standard test of means was performed to determine significant variance between populations ($P \leq 0.05$).

### 2.3. Molecular identification

#### 2.3.1. DNA extraction and amplification

DNA was extracted from one single cyst for each population using the protocol of Waeyenberge et al. (2000) with some modifications. PCR reactions were performed in a total volume of 50 µL containing 1 µL of nematode DNA, 25 µL of 2X Dream Taq PCR Master Mix (Fermentas Life Sciences, USA), 22 µL of $ddH_2O$, and 1 µM each of forward primer AB28 (5′-CGTAACAAGGTAGCTGTAG-3′) and reverse primer TW81 (5′-TCCTCCGCTAAATGATATG-3′) (Joyce et al., 1994).

Reaction steps consisted of an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. The program finalized at 72 °C for 10 min. A negative control (no DNA) was used to ensure that there was no contamination in amplifications. Amplification quality was evaluated using UV illumination and ethidium bromide staining on 1.5% agarose gel (100 V, 40 min).

**Table 1.** Agroecological regions where sequenced *Heterodera latipons* samples were collected from wheat and barley fields

| No. | Province | District | Location | Species | Accession number |
|---|---|---|---|---|---|
| 1 | Adana | Sarıçam | Giriş Gediği | *H. latipons* | KY352355 |
| 2 | Adana | Sarıçam | Kepeztepe | *H. latipons* | KY352356 |
| 3 | Adana | Sarıçam | Dutluca | *H. latipons* | KY352357 |
| 4 | Adana | Sarıçam | Tülüler | *H. latipons* | KY352358 |
| 5 | Adana | Karaisalı | Yazıbaşı | *H. latipons* | KY352359 |
| 6 | Adana | Karaisalı | Salbaş | *H. latipons* | KY352360 |
| 7 | Adana | Karaisalı | Hacılar | *H. latipons* | KY352361 |
| 8 | Adana | Sarıçam | Torunlar | *H. latipons* | KY352362 |
| 9 | Hatay | Kırıkhan | Kurtlusoğuksu | *H. latipons* | KY352363 |
| 10 | Hatay | Kırıkhan | Merkez I | *H. latipons* | KY352364 |
| 11 | Hatay | Kırıkhan | Merkez II | *H. latipons* | KY352365 |
| 12 | Hatay | Reyhanlı | Karakaya | *H. latipons* | KY352366 |
| 13 | Hatay | Reyhanlı | Müşrüfe | *H. latipons* | KY352367 |
| 14 | Hatay | Kırıkhan | Mazmanlı | *H. latipons* | KY352368 |
| 15 | Hatay | Kırıkhan | Beyaz-Bestami | *H. latipons* | KY352369 |
| 16 | Hatay | Kırıkhan | İmece | *H. latipons* | KY352370 |
| 17 | Gaziantep | Karkamış | Türkyurdu | *H. latipons* | KY352371 |
| 18 | Gaziantep | Karkamış | Akçaköy I | *H. latipons* | KY352372 |
| 19 | Gaziantep | Karkamış | Akçaköy II | *H. latipons* | KY352373 |
| 20 | Gaziantep | Karkamış | Arıkdere I | *H. latipons* | KY352374 |
| 21 | Gaziantep | Karkamış | Arıkdere II | *H. latipons* | KY352375 |
| 22 | Gaziantep | Karkamış | Merkez | *H. latipons* | KY352376 |
| 23 | Gaziantep | Karkamış | Sınır I | *H. latipons* | KY352377 |
| 24 | Gaziantep | Karkamış | Sınır II | *H. latipons* | KY352378 |
| 25 | Gaziantep | Oğuzeli | Karaman | *H. latipons* | KY352379 |
| 26 | Gaziantep | Oğuzeli | Anavatan I | *H. latipons* | KY352380 |
| 27 | Gaziantep | Oğuzeli | Anavatan II | *H. latipons* | KY352381 |
| 28 | Kilis | Musabeyli | Haydarlar | *H. latipons* | KY352382 |
| 29 | Kilis | Musabeyli | Deliçay | *H. latipons* | KY352383 |
| 30 | Kilis | Musabeyli | Besenli | *H. latipons* | KY352384 |
| 31 | Kilis | Merkez | Acar | *H. latipons* | KY352385 |
| 32 | Kilis | Merkez | Karaöner | *H. latipons* | KY352386 |
| 33 | Kilis | Merkez | Yığmatepe | *H. latipons* | KY352387 |
| 34 | Kilis | Elbeyli | Doğanlı | *H. latipons* | KY352388 |
| 35 | Kilis | Elbeyli | Çıldıroba I | *H. latipons* | KY352389 |
| 36 | Kilis | Elbeyli | Çıldıroba II | *H. latipons* | KY352390 |
| 37 | Mardin | Nusaybin | Merkez | *H. latipons* | KY352391 |
| 38 | Mardin | Nusaybin | Cizre Yolu | *H. latipons* | KY352392 |
| 39 | Mardin | Nusaybin | Yolindi | *H. latipons* | KY352393 |
| 40 | Mardin | Nusaybin | Gölova | *H. latipons* | KY352394 |
| 41 | Mardin | Kızıltepe | Güneyli | *H. latipons* | KY352395 |
| 42 | Mardin | Kızıltepe | Şenyurt Sınır | *H. latipons* | KY352396 |

**2.3.2. Sequencing and phylogenetic tree**

Gene Research and Biotechnology (Ref-Gen), Ankara, Turkey, sequenced PCR products from each sample in both directions to obtain overlapping sequences of both DNA strands. A total of 42 DNA sequences were screened using a blast search for their statistical similarities (positive matrix scores) to ITS-rRNA gene sequences of identified nematodes in GenBank (www.ncbi.com). Based on ITS-rDNA sequences, all tested populations were identified as *H. latipons* (Table 1). Sequence traces were quality-checked using the Trace Editor of MEGA v 7.0. (Kumar et al., 2016). The alignments of these DNA sequences were conducted with Clustal X using the default parameters for gap opening and gap extension penalties (Kimura, 1980). All aligned characters were applied in the phylogenetic analysis. Evolutionary history was inferred using the maximum likelihood method, based on the Kimura 2-parameter model (Kimura, 1980). Gaps were treated as missing data. *Cryphodera brinkmani* (Nematoda: Heteroderidae) (accession number: AF274418) was used as an outgroup to root the trees and for character polarization. Bootstrap support was calculated for all analyses using 1000 replicates.

## 3. Results

### 3.1. Morphological and morphometric identification

All 42 cyst nematode populations were identified as *H. latipons* based on the similar morphological and morphometric characteristics of cysts and J2, though morphometric data analysis revealed some variability among the populations studied (Tables 2 and 3). An example of the morphological characteristics of *H. latipons* second stage juveniles and their structures is presented in Figure 1.

The robust J2 stylet is bowl-shaped with basal knobs (Figure 2). The *H. latipons* J2 has a relatively symmetrical head with a narrow, rounded tail tip (Figure 3). Due to the variation exhibited in the morphological measurements and morphometric characteristics, *H. latipons* populations were divided into two groups based on the statistical analysis (Group I and Group II). Group I comprised the populations from Adana and Hatay provinces, whereas the populations from Gaziantep, Kilis, and Mardin provinces formed Group II. Both the tail and hyaline portion differed significantly in Group I, being slightly shorter, at 53–54 µm vs. 70–73 µm and 29–33 µm vs. 42–43 µm, respectively (Figures 2 and 3). The tail tip of Group II was less pointed than that of Group I. Similarly, all morphometric characteristics of J2 showed some variation between Group I and Group II (Table 2). ANOVA revealed significant differences in J2 body length, c' ratio, body size, stylet dimension, body size at the anus, tail size, and hyaline tail distance. No differences were observed in the a and c ratios. J2 stylet length and knob shape (Table 2) were similar to those previously reported by Handoo (2002) and Subbotin et al. (2003).

In general, cysts from all populations varied in size and were mostly lemon-shaped (Baldwin and Mundo-Ocampo, 1991), with a prominent and bifurcated vulval slit on both sides. *H. latipons* cysts differed statistically from other *Heterodera* species cysts by having a strong and deep underbridge without bullae and greater fenestral length (51–76 µm vs. 35–62 µm), larger vulval bridge width (19–34 µm vs. 9–15 µm), and longer vulval slit length (5.3–11 µm vs. 7.3–13.4) (Table 3). *H. latipons* cysts of Group I populations shared similar morphological features, e.g., a round semifenestra divided by a detachment lengthier than the semifenestra size, and a relatively characteristic underbridge located in the middle of the vulval section (Figure 4). Group I cysts were lighter with a less pointed cone tip. Furthermore, the vulval slit of Group II cysts was shorter (3.75 µm) than those of Group I (7.1 µm) (Figure 4). However, cysts of Group I have higher vulval slit values (<8.8 µm) than those haplotype species encompassed within the other *Heterodera* species (Baldwin and Mundo-Ocampo, 1991). Compared to Group I, Group II cysts have a wider underbridge with more sclerotization (Figure 4), while J2 stylet knobs are more anteriorly concave in Group I compared to Group II.

### 3.2. Molecular identification

The rDNA-ITS regions of all 42 nematode populations were successfully amplified using the primers described above. For all populations, amplification of the ITS-rDNA region (ITS1-5.8S-ITS2), including the flanking parts of the 18S and 28S genes, yielded a single fragment of approximately 1060 bp. No PCR products were obtained in the negative control that lacked a DNA template (Figure 5). Moreover, 42 sequences from the surveyed populations of cyst nematodes were attained and identified as *H. latipons*. One overall consensus sequence was derived from these sequences and was used for further phylogenetic analysis. The corresponding 18S rRNA gene sequences of these nematode isolates matched 42 nematode species listed in the GenBank database (Table 1).

A phylogenetic tree clustering the populations at different levels based on genetic distance was constructed from the ITS sequence alignment (Figure 6). Samples from five geographically distant sites were evaluated to cluster 42 *H. latipons* populations and one outgroup (*Cryphodera brinkmani* AF274418). The phylogenetic relationship of *H. latipons* populations was compared to international genotypes. The phylogenetic tree was generated from 1000 bootstrapped sequence alignments, which were subjected to global rearrangement with random replications. According to this tree, a clean separation of the outgroup of *H. latipons* was found. Species with bootstrap values of over

**Table 2.** Morphological and morphometric characteristics of J2s of *Heterodera latipons* populations.

| Characteristic (measured in µm) | Adana | Hatay | Kilis | Gaziantep | Mardin |
|---|---|---|---|---|---|
| | 10 individual J2s | 10 individual J2s | 10 individual J2s | 10 individual J2s | 10 individual J2s |
| | Mean ± std. error (min–max) | Mean ± std. error (min–max) | Mean ± std. error (min–max) | Mean ± std. error (min–max) | Mean ± std. error (min–max) |
| Body length (L) | 570.8 ± 43.48 (499–627) | 632.8 ± 6.01 (602–659) | 503.2 ± 13.51 (479–519) | 547.23 ± 15.51 (527–566) | 540.2 ± 31.2 (469–581) |
| Body width | 26.3 ± 2.31 (23–32) | 24.48 ± 1.9 (22–27.7) | 21.4 ± 1.02 (20–23) | 22 ± 0.7 (21–23) | 27.2 ± 3.1 (23–32) |
| Esophagus length | 128.8 ± 10.11 (103.5–148) | 125.2 ± 11.1 (110–140) | 100.4 ± 4.128 (95–105) | 113.25 ± 2.1 (107–117) | 102.2 ± 6.5 (91–131) |
| Stylet knobs width | 5.77 ± 0.4 (5–8) | 6.46 ± 0.2 (6–7) | 4.86 ± 0.86 (3.8–7.6) | 4.32 ± 0.34 (3.7–5.8) | 4 ± 0.3 (3.5.5–6) |
| Stylet length | 22.6 ± 0.75 (20–25.2) | 24.7 ± 1.07 (23–26) | 21,3 ± 0.5 (18–23) | 21 ± 0.25 (19–23) | 20 ± 1.2 (17–26) |
| Labial region height | 5.66 ± 0.42 (4–6.7) | 54 ± 0.35 (52–56) | 4.92 ± 0.65 (4–6) | 4.87 ± 0.74 (4–6) | 5.3 ± 0.6 (4.5–6) |
| Labial region width | 9.87 ± 0.29 (8.6–12) | 9.2 ± 0.4 (8–11) | 11.4 ± 0.6 (10–12) | 10.5 ± 0.44 (10–11) | 10.34 ± 0.6 (9.7–11) |
| DEGO | 5.67 ± 0.17 (4.7– 6.3) | 5.16 ± 0.26 (4–6) | 6.04 ± 0.86 (5–7.5) | 6.3 ± 0.22 (6–6.9) | 5.4 ± 0.6 (4.5–6) |
| Median bulb width | 14.1 ± 1.8 (10–18) | 13 ± 1.67 (10–15) | 12.8 ± 0.98 (11–14) | 11.75 ± 0.12 (11–13) | 10.6 ± 1.2 (9–15) |
| Median bulb height | 18.2 ± 2.4 (15–22) | 16.2 ± 1.1 (13.6–18) | 16.6 ± 2.154 (14–20) | 17.5± 1.11 (16–19) | 20.6 ± 2.1 (17–23) |
| Distance from anterior end to median bulb (MBV) | 77.2 ± 3.19 (64.6–83) | 85.2 ± 7.14 (74–96) | 63 ± 3.9 (58–68) | 72.25 – 3.16 (65–78) | 71.2 ± 4.4 (66–89) |
| Distance from anterior end to excretory pore (Mb) | 127 ± 6.64 (110–146) | 127.8 ± 7.2 (120–137) | 104.6 ± 0.93 (102–113) | 116.25 ± 2.76 (109–121) | 106.2 ± 2.3 (102–127) |
| Pharynx | 57.6 ± 4.45 (47–64) | 58.4 ± 8.71 (44–68) | 46.4 ± 1.03 (43–50) | 52.75 ± 0.3 (51–54) | 58.8 ± 4.4 (49–71) |
| Tail length | 66.2 ± 4.8 (52–74) | 73.6 ± 2.33 (71–78) | 53.6 ± 3.39 (39–57) | 63.75 ± 4.8 (57–70) | 50.4 ± 6.4 (41–74) |
| Body width at anus | 14.94 ± 1.75 (11.6–24) | 14.8 ± 0.75 (12–19) | 18.6 ± 1.06 (13–21) | 17 ± 1.87 (15–20) | 18.2 ± 0.8 (17–19) |
| Hyaline region length (H) | 42.5 ± 2.03 (25–48) | 42 ± 2.4 (38–45) | 29.6 ± 1.02 (26–33) | 33 ± 2.55 (30–37) | 33.2 ± 1.9 (28–50) |
| a (body length/body width) | 20.2 ± 1.06 (16.28–22.65) | 25.9 ± 1.29 (23.79–27.36) | 18.5 ± 1.2 (17.2–25) | 20.89 ± 2.99 (13.95–26.57) | 19.86 ± 3.3 (16.3–28.8) |
| b (body length/esophagus length) | 4.05 ± 0.31 (3.66–5.03) | 5.75 ± 0.48 (4.92–6.43) | 4.01 ± 0.13 (3.29–5.26) | 4.83 ± 0.2 (4.52–5.07) | 4.42 ± 0.2 (4–5.4) |
| c (body length/tail length) | 8.59 ± 0.66 (7.24–9.95) | 8.6 ± 0.28 (8.25–9.03) | 6.98 ± 0.64 (4.77–10.5) | 6.58 ± 0.46 (4.97–9.25) | 5.67 ± 1.1 (4.2–10.7) |
| c’ (tail length/anus diameter) | 3.73 ± 0.31 (2.58–4.70) | 4.14 ± 0.14 (3.94–4.29) | 3.67 ± 0.27 (3.29–4.07) | 3.76 ± 0.2 (3.5–4.13) | 3.86 ± 0.4 (3.2–4.3) |
| L/MBV (body length/distance anterior end to median bulb) | 7.28 ± 0.56 (6.49–8.15) | 7.47 ± 0.33 (7.13–8.6) | 5.98 ± 0.18 (4.23–8.75) | 6.57 ± 0.12 (5.15–7.36) | 6.65 ± 0.18 (6–6.96) |
| Body length/median bulb width | 42.68 ± 5.15 (34.28–49.90) | 48.6 ± 2.6 (43–62.20) | 39.31 ± 1.05 (36.85–45.45) | 36.57 ± 2.8 (33.54–50.73) | 39.72 ± 2.9 (33.1–50.9) |
| Hyaline region length/stylet length | 1.60 ± 0.26 (1.09–1.94) | 1.7 ± 0.05 (1.64–1.75) | 1.32 ± 0.05 (1.24–1.39) | 1.5 ± 0.17 (1.3–1.76) | 1.8 ± 0.2 (1.5–2.08) |

**Table 3.** Morphological and morphometric cyst characteristics of *Heterodera latipons* populations.

| Characteristic (measured in µm) | Adana | Hatay | Kilis | Gaziantep | Mardin |
|---|---|---|---|---|---|
| | 8 individual cysts | 8 individual cysts | 8 individual cysts | 8 individual cysts | 8 individual cysts |
| | Mean ± std. error (min–max) values) | Mean ± std. error (min–max) values) | Mean ± std. error (min–max) values) | Mean ± std. error (min–max) values) | Mean ± std. error (min–max) |
| Length | 806.12 ± 18.22 (759–834) | 754.75 ± 23.83 (697–839) | 682 ± 48.1 (601–763) | 582.5 ± 10.2 (556–965) | 746.5 ± 12.04 (714–772) |
| Width | 588.15 ±14.7 (526–623) | 577.25 ± 17.7 (529–620) | 407.5 ± 10.5 (367–578) | 483.5 ± 6.8 (398–567) | 504.25 ± 15.22 (468–559) |
| Length/width | 1.37 ± 0.04 (1.22–1.46) | 1.31 ± 0.06 (1.22–1.37) | 1.03 ± 0.03 (0.98–1.37) | 1. 07 ± 0.12 (0.64–1.7) | 1.08 ± 0.05 (0.96–1.49) |
| Vulval slit length | 7.20 ± 0.8 (6.4–8) | 7.10 ± 1.34 (5.6–8.8) | 5 ± 1 (4–8) | 5.25 ± 0.35 (4.9–6) | 3.95 ± 0.5 (2–10) |
| Fenestral length | 68.5 ± 0.5 (68–69) | 67.88 ± 4.1 (42.5–74) | 53.5 ± 1.5 (51–55) | 49.5 ± 0.5 (49–50) | 70,5 ± 0.07 (70–71) |
| Fenestral width | 23 ± 2 (17–26) | 22.63 ± 2.57 (14.7–26.3) | 18.5 ± 1.5 (16–23) | 22 ± 2.2 (17–26) | 22.25 ± 2.36 (17–35) |
| Vulval bridge width | 21 ± 0.1 (20–22) | 22.65 ± 1.44 (19.6–28.8) | 9.50 ± 0.5 (9–10) | 12.50 ± 1.5 (8.9–16.1) | 12.5 ± 2.24 (8–24) |
| Underbridge | Present | Present | Present | Present | Present |
| Underbridge length | 104 ± 4 (97–110) | 99.75 ± 3.7 (65–109) | 52.60 ± 2.80 (48–56) | 55.5 ± 2.8 (50–62) | 68.12 ± 1.32 (66–72) |

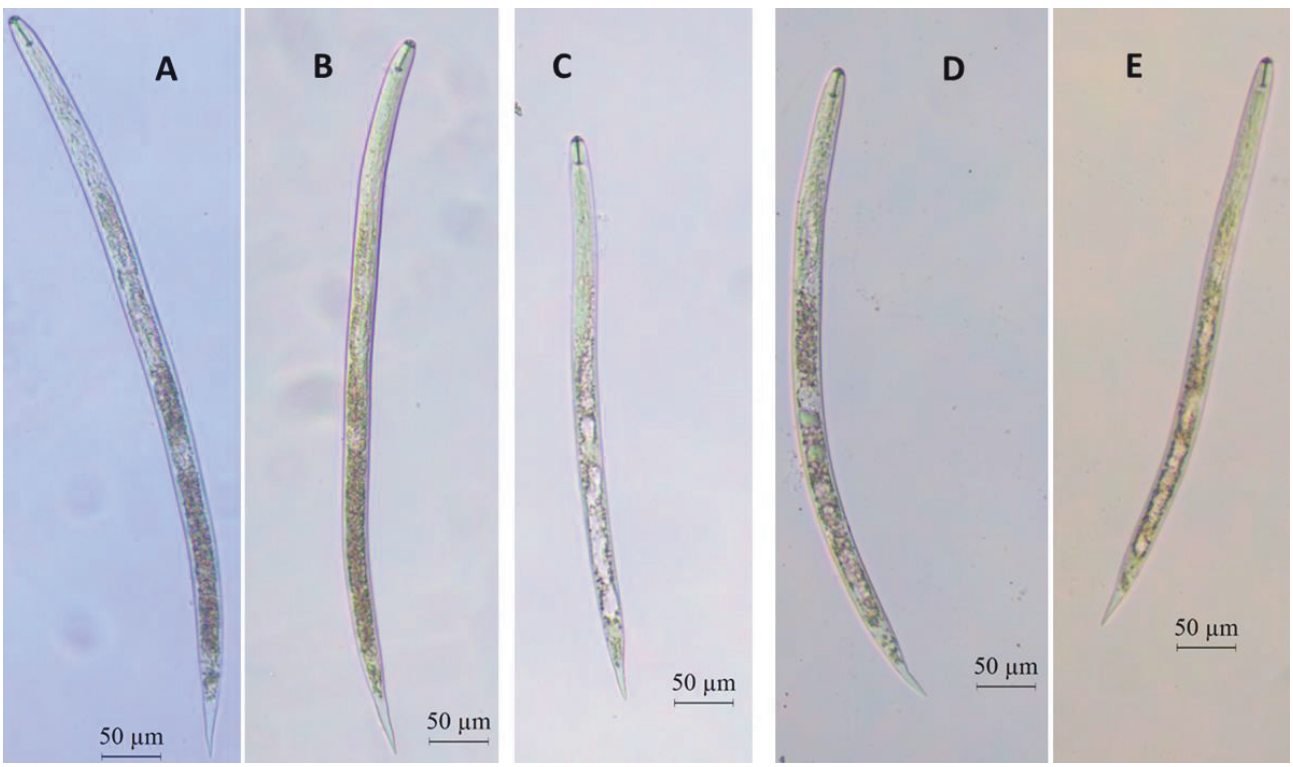


**Figure 1.** Second stage juveniles (J2s) of *Heterodera latipons* found in Adana (A), Hatay (B), Kilis (C), Gaziantep (D), and Mardin (E).

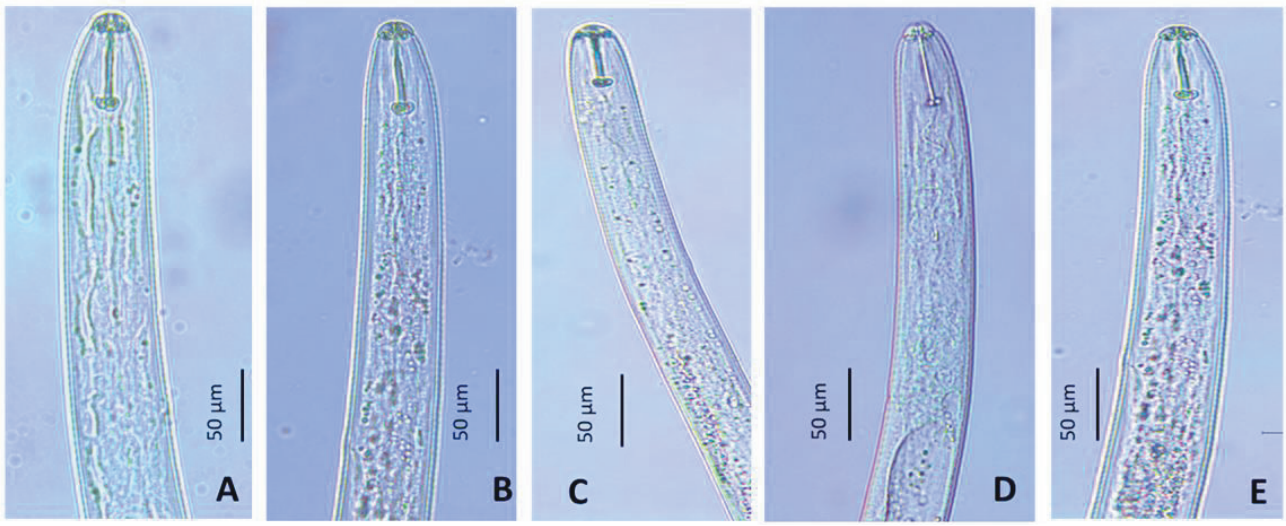


**Figure 2.** Anterior regions of *Heterodera latipons* J2s found in Adana (A), Hatay (B), Kilis (C), Gaziantep (D), and Mardin (E).

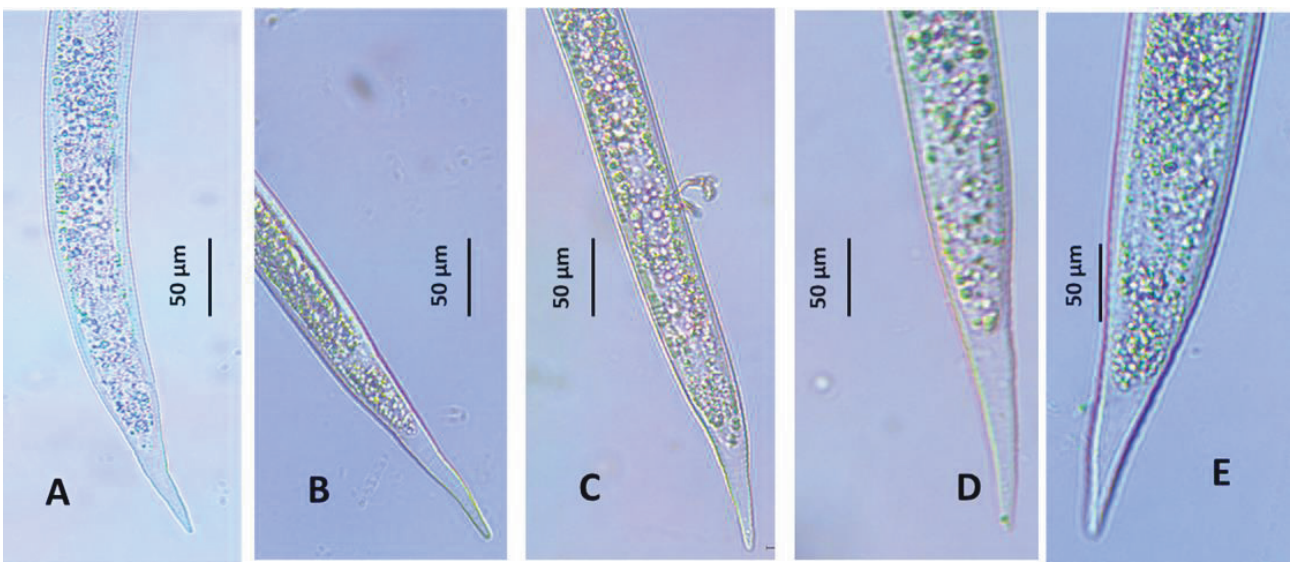


**Figure 3.** Tail shape of *Heterodera latipons* J2s found in Adana (A), Hatay (B), Kilis (C), Gaziantep (D), and Mardin (E).

60% are given. Results indicated that the ribosomal DNA sequences of the 42 *H. latipons* isolates were 60% similar to each other, meaning that intraspecific polymorphism exists among the nematode populations. This conclusion is backed up by the ITS-rDNA sequences. Species within the *H. latipons* population were clearly divided into two groups (Group I and Group II) within the phylogenetic tree (Figure 6).

Group II included mainly Gaziantep, Kilis, and Mardin *H. latipons* populations (from KY352355 to KY352370), along with *H. latipons* from Cyprus (JX024187), whereas Group I included mostly Adana and Hatay *H. latipons* populations, along with *H. latipons* populations KP708720 from Turkey. Some samples in Group II, such as 33, 38, 39, and 41, are very close to the Syrian (JX024177) and Jordanian (JX024188) *H. latipons* populations, while other samples of same group such as 18, 24, 34, and 35 are very close to the Syrian (JX024181–JX024182) and Iranian (JX024189) populations. The rDNA-ITS sequences of Group II populations were 98% similar to the sequences of *H. latipons* published in GenBank from Cyprus (JX024187), Syria (East Region) (AF274402), Morocco (JQ319037), and Jordan (JX024188), suggesting that they might all be the same pathotype. Moreover, samples 5, 6, 11, and 12 in Group I are alongside those from Syria (JX024176, JX024178, and JX024180) (Figure 6). The

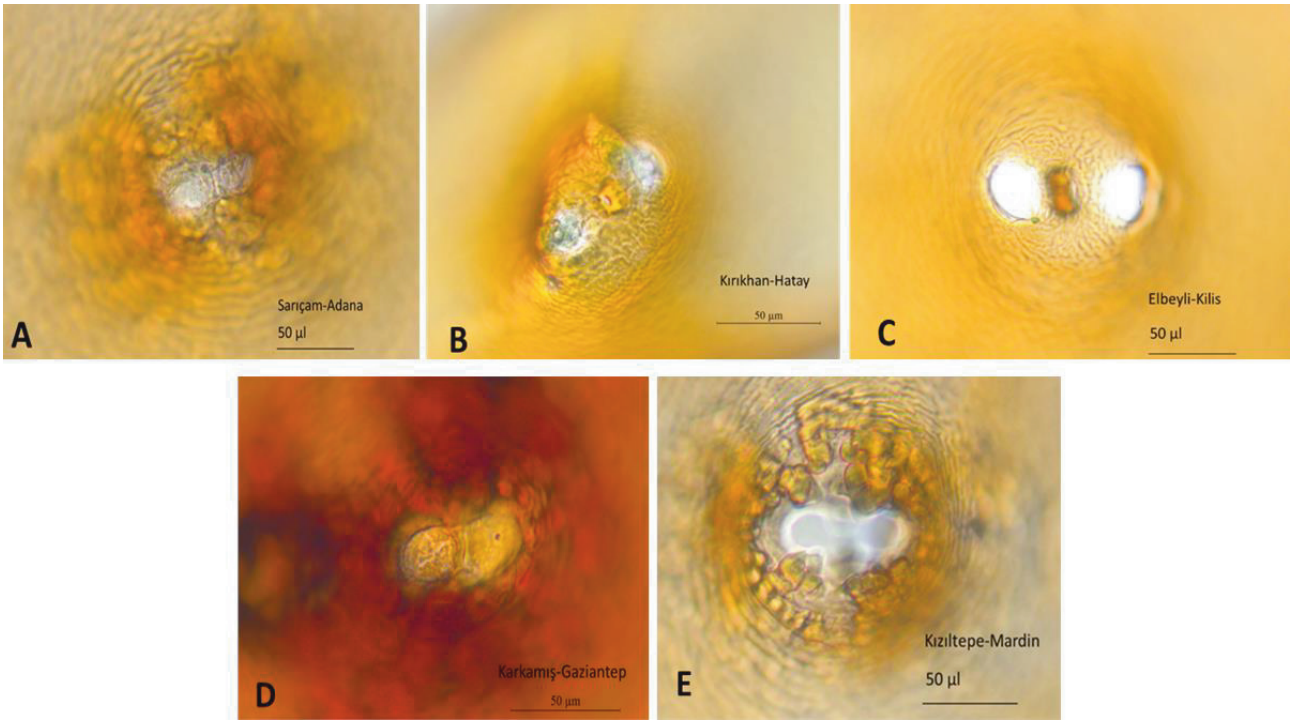


**Figure 4.** Fenestral area of *Heterodera latipons* vulval cones from Adana (A), Hatay (B), Kilis (C), Gaziantep (D), and Mardin (E).


**Figure 5.** PCR products of *Heterodera latipons* using AB28 and TW81 universal primers yielded a single fragment of approximately 1060 bp. L: 100-bp DNA ladder; C: negative control.

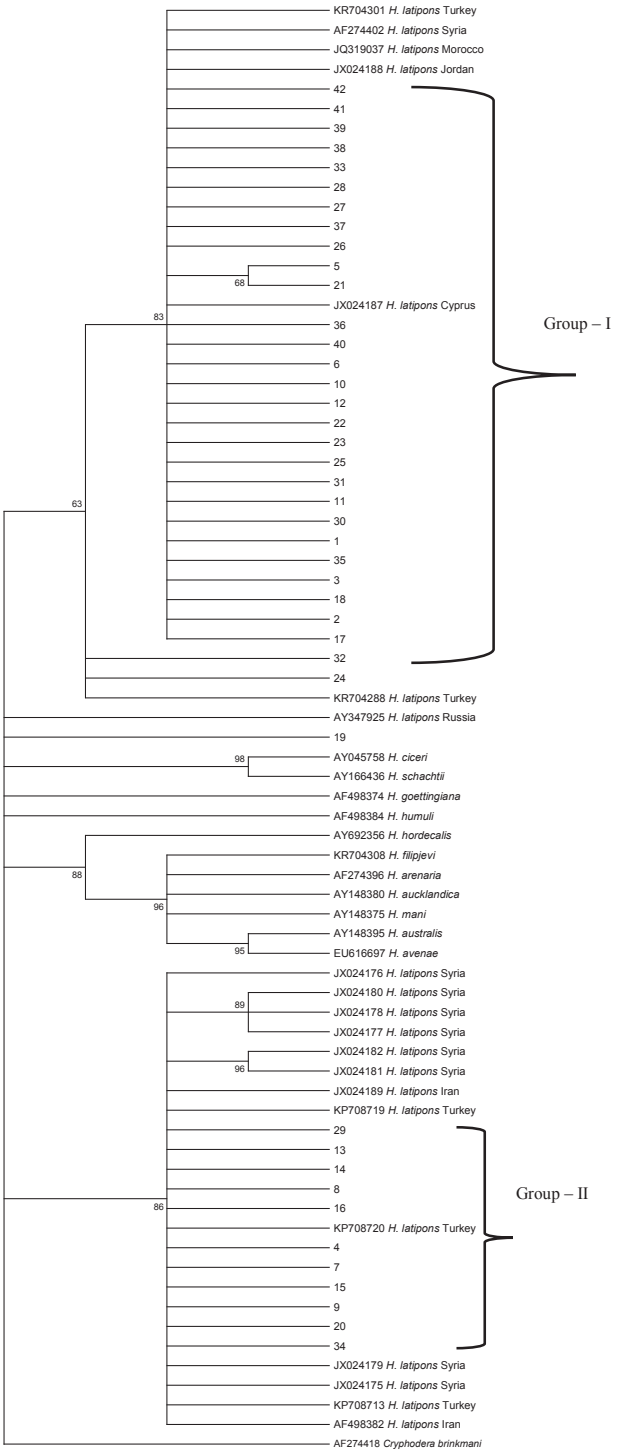


**Figure 6.** Phylogenetic tree (maximum likelihood) constructed through the ITS sequence alignment from 42 populations of *Heterodera latipons*. Bootstrap values (more than 60%) are given for the appropriate clades. Populations are designated with the code described in Table 1.

sequences of Group I populations were 98% similar to the rDNA sequence of an isolate from Syria (West Region), suggesting that they might be the same species pathotype.

There was high (>99%) sequence similarity among *H. latipons* isolates, indicating the common ancestry of the species in Turkey. However, one population from Gaziantep

(number 19) was slightly different from the Cyprus, Syrian, Iranian, and Jordanian genotypes recorded in GenBank.

## 4. Discussion

This study aimed to characterize *H. latipons* in Turkey and contribute to the global species database. The combination of morphological and molecular data provided convincing evidence to identify a population of *H. latipons* as reported in previous studies (Subbotin et al., 2003; İmren et al., 2012; Toktay et al., 2015).

Cyst nematodes' morphology is complicated by their sexual dimorphism, sedentary lifestyle of the female, and presence of a dormant cyst (Agrios, 1997). Multiple development stages are important in their morphology and useful for identification, and cysts and juveniles are of the greatest value and the most widely used (Golden, 1986). *Heterodera* species form cysts with a posterior protuberance, the terminal or vulval cone (Fleming and Powers, 1998). However, cyst size and shape are variable, and genera/species identification is more reliable when based on other characteristics of the genus *Heterodera* (Subbotin et al., 2003).

Morphological and morphometric cyst characteristics enable *H. latipons* to be differentiated from other *Heterodera* species (Handoo, 2002). Useful characteristics for distinguishing *H. latipons* are the presence of a strong underbridge in the vulval cone and a few bullae situated below the fenestrae of cysts (Subbotin et al., 2003). *H. latipons* could also be readily identified using either the fenestral length or the vulval bridge width of the cyst, and either tail length or the hyaline part of the juvenile tail (Wouts et al., 1995). In this study, separating specimens based on bullae development and presence of an underbridge was successful at low magnification. Juveniles are the second choice in identifying cyst nematodes species (Golden, 1986). The analysis of the juvenile stylet may enable differentiation of several *Heterodera* species. J2 stylet length and the hyaline part of the tail are the more reliable juvenile characteristics that can be measured and used to identify *H. latipons* (Hando et al., 2002).

This study identified 42 *H. latipons* populations based on the morphological features of cysts and J2s with 17 morphometric characteristics (Tables 2 and 3). These *H. latipons* populations were grouped into two groups: Group I (populations from Adana and Hatay provinces) and Group II (populations from Gaziantep, Kilis, and Mardin provinces). Variation between Group I and Group II was determined by cyst body size, as well as midbody width, body thickness at anus, midbody width, head height, b ratio, c ratio, c' ratio, and caudal ratio A. Previous studies have also used morphological and morphometric characteristics of cysts and J2s to identify *H. latipons* populations (with some intraspecies variation) in Turkey's Mediterranean and Central and Eastern Anatolian Regions (Abidou et al., 2005; Toktay et al., 2015). Handoo (2002) reported that some cyst nematode populations have intraspecific diagnostic characteristics that make them distinguishable among each other. It has also been reported that geographical distribution, ecophenotypic effects, environmental and nutritional factors, and plant host are factors that can affect morphometric differences (Subbotin et al., 2003).

Nucleotide sequences of rDNA are highly useful for identifying cyst nematode species and examining their phylogenetic relationships together with other *Heterodera* species (Subbotin et al., 2010). Handoo (2002) reported that DNA sequencing is an accurate tool for identifying closely related *Heterodera* species with highly similar morphology and morphometric characteristics. Species within the *H. latipons* population were clearly divided into two groups (Group I and Group II) in the phylogenetic tree (Figure 6). Furthermore, *H. latipons* populations showed intraspecific polymorphism based on the ITS-rDNA sequences. Madani et al. (2004) and Rivoal et al. (2003) also demonstrated intraspecific variation between *H. latipons* populations. Likewise, İmren et al. (2012) observed genetic variation in limited specimens of *H. latipons* in Turkey's Mediterranean Region. However, Toktay et al. (2015) did not detect any genetic variation in three populations of *H. latipons* in the East Anatolian Region of Turkey.

This study demonstrates the importance of some cyst and J2 body dimension characteristics with sequences of rDNA that can be used to identify diverse populations of *H. latipons* and define intraspecific variation within populations. This study provides morphological, morphometric, and molecular data about *H. latipons* populations in Turkey and indicates the presence of intraspecific variation. Phenotypic and genotypic differences among *H. latipons* populations may cause heterogeneity, with possibly two or more pathotypes. Detailed and comprehensive pathotype studies of *H. latipons* from different ecological regions of Turkey are therefore needed to determine the locations of *H. latipons* pathotypes in existence. The results will be vital in enabling Turkish breeding programs to identify novel sources of resistance in wheat germplasm targeted to *H. latipons* populations.

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