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**MAHMOUDI NILOUFAR**

# Comparative morphometric analysis of nematodes *Ditylenchus destructor* Thorne, 1945 populations of Iranian and Russian origin and development of new test systems for their molecular genetic identification

Specialty 4. 1. 3 Agro chemistry, Agro soil science, Plant protection and Quarantine plants

Thesis for the candidate degree of biological sciences

Scientific Advisor:
Pakina Elena Nikolaevna
Doctor of Agricultural Sciences, Associate Professor

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ФЕДЕРАЛЬНОЕ ГОСУДАРСТВЕННОЕ АВТОНОМНОЕ ОБРАЗОВАТЕЛЬНОЕ УЧРЕЖДЕНИЕ ВЫСШЕГО ОБРАЗОВАНИЯ
«РОССИЙСКИЙ УНИВЕРСИТЕТ ДРУЖБЫ НАРОДОВ»

*На правах рукописи*

**МАХМУДИ НИЛУФАР**

# Сравнительный морфометрический анализ нематод *Ditylenchus destructor* Thorne, 1945 популяций иранского и российского происхождения и разработка новых тест-систем для их молекулярно-генетической идентификации

Специальности 4.1.3 Агрохимия, агропочвоведение, защита и карантин растений

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Пакина Елена Николаевна
доктор сельсхозяйственных наук, доцент

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## Contents

# INTRODUCTION

**Relevance of the topic.** Crops can be attacked by a variety of diseases and pests, which significantly reduce crop yields and economic production. Plant-parasitic nematodes, together with fungus, viruses, and bacteria, are the four principal plant pathogenic microorganisms that can harm farmed crops and significantly reduce yields [1]. Entirely dependent on plant cells for nutrition, plant-parasitic nematodes can partially or fully complete their life cycles in soil or plant tissues.

Parasitic nematodes have the potential to harm any plant species, reducing output or quality. According to estimates, the annual yield loss of crops brought on by nematode pest's amounts to around 5% of the world's food production, which is worth between USD 125 and USD 157 billion [2]. However, given that, many farmers, particularly those in developing nations, are unaware of the effects of these pests [3], noted that this level of loss is anticipated to be significantly higher. Because of this, the average yield loss in some crops might be as high as 20% per year [4].

*Ditylenchus* species can be found in a wide range of ecological niches and have a wide range of dietary preferences. The majority of the *Ditylenchus* species are soil-dwelling, free-living nematodes that eat fungus, whereas others are unavoidable parasites of higher plants [5].

Symptoms caused by the nematode of the stem and bulb vary based on the host and the type of tissue affected. Whereas there are very few physical differences among all plant parasitic *Ditylenchus* species and they are all fairly similar to one another [1, 6].

*Ditylenchus dipsaci and Ditylenchus destructor* are two of the most significant plant-parasitic nematode species found in the Eurasian region. *Ditylenchus dipsaci and Ditylenchus destructor* share a lot of similarities morphologically, although they differ pathogenetically [7, 8].

With approximately 20 different biological races, diverse host ranges, the occurrence of reproductive isolation, various stages of speciation, *Ditylenchus dipsaci* exhibits substantial intraspecific variation [3, 9]. Because of this, it is challenging to identify this species [10, 11]. Nematode taxonomists are therefore increasingly

requesting that these nematodes be evaluated with a variety of objectives. Due of the heterogeneity in field populations, proper detection and identification of *Ditylenchus destructor* are crucial.

Nematode identification using various molecular methods, as opposed to morphometric data, offers the precise and speedy species identification required for the inquiry. For *Ditylenchus destructor*, the European and Mediterranean Organization for Plant Protection (EPPO) offers a diagnostic technique [12]. The guideline suggests using both genetic and morphometric methods to identify both nematode species. The management and risk assessment of these nematodes can benefit from the utilization of the data generated from these methods.

In order to distinguish between plant-parasitic nematodes, genomic DNA fragments were examined using restriction enzymes in the first report of a DNA approach used for taxonomic purposes [13]. When there were only tiny amounts of material available, discrimination was made possible by the development of the polymerase chain reaction (PCR) [14].

The three Ss of PCR are selectivity, sensitivity, and speed [15, 16]. It is possible to identify genetic variations that can be used or modified for taxonomic and diagnostic reasons using a variety of DNA-based approaches [5, 9, 17, 18]. A particularly efficient method of identifying inter- and intra-specific changes between genera and species, as well as within species, is the amplification of certain genomic areas.

For taxonomic and diagnostic reasons, ribosomal DNA (rDNA) and mitochondrial DNA contain the two most often repeated sections (mtDNA). fragment length polymorphisms restrictions were utilized by [5] to differentiate between *D. destructor* and *D. africanus* because polymerase chain reaction (PCR) technology is sensitive enough to resolve variations between closely related genera.

Ribosomal DNA (rDNA) and mitochondrial DNA comprise the two most frequently repeated regions for taxonomic and diagnostic purposes (mtDNA). Because polymerase chain reaction (PCR) technology is sensitive enough to resolve changes between closely related taxa, fragment length polymorphisms restrictions were used by [19], to distinguish between *D. destructor* and *D. africanus*.

The study of the ITS-rDNA region, which is a remarkably conserved taxonomic identifier for many genera of worms, is particularly significant in the field of molecular diagnostics and is becoming more and more popular. The ITS1 region of *D. dipsaci* was first sequenced by [20], however more than 50 sequence accessions of rRNA fragments from *D. destructor* derived from samples collected from various locations and host plants are currently accessible in the GenBank database.

At present, many potato farmers have 1000–5000 hectare of potato fields. Potato seed quality management, improper crop rotation, and a lack of appropriate logistical technologies are common problems these farms encounter. As a result, since 2010, nematodes have been spreading more widely and causing more harm overall in the Russian Federation.

According to [1, 21], about 40,000 hectares of commercial potatoes are currently heavily infested with *Ditylenchus destructor*. As a rule, 5–10% of infected tubers are found in the harvested crop. The use of irrigation systems resulting in persistently high levels of soil moisture can exacerbate infection, thereby increasing the percentage of infected tubers by 10% or more. Individual farmers lost roughly 30% of their crops between 2015 and 2019 because of *D. destructor* nematode infesting fields in Russia's Central and Volga regions [22, 23].

**The purpose of research.** The main source of the appearance of *Ditylenchus* in the fields is infected planting material. The use of phytoparasite-free seed potatoes reduces the infection load in the fields. To reduce the risk of infection in new fields, seed tubers should be pre-tested.

An important component in this process is the exact species identification of *Ditylenchus* species on potatoes.

In this regard, an urgent problem is the development of fast and accurate molecular test systems for the identification of harmful species of stem and rot nematodes.

**Purpose and objectives of the study.** The currently known molecular methods for identifying *D. destructor*, in contrast to the closely related species *D. dipsaci*, are not entirely reliable, since they do not always give a stable result. In this regard, the purpose

of this work was to study geographically distant populations of *D. destructor*, and on the basis of these data to develop a new diagnostic PCR primers, as well as to test and improve the species-specific diagnostic method

To achieve this goal, the following tasks were set:

1. Obtain and analyze the morphometric variability of *D. destructor* populations from Russia and Iran.
2. To study the genetic variability of *D. destructor* populations.
3. Develop two sets of pairs of new species-specific primers for molecular diagnostics of *D. destructor*.
4. Test for primer selectivity.
5. Test for the sensitivity of primers.

**The scientific novelty of the work:**

1. Morphological and genetic comparison of *D. destructor* individuals between populations from Russia and Iran was carried out for the first time.
2. Two sets of new species-specific primer pairs have been developed for molecular diagnostics of *D. destructor* populations from Russia and Iran.
3. Tests for sensitivity and selectivity of primers showed high efficiency of new primers dsn.1 F/R and dsn.2F/R for identification of *D. destructor*.

**Theoretical and practical significance.** Is to develop new primers sets. Two pairs of primers, dsn.1 F/ R and dsn.2 F/ R, have been developed. Their analytical sensitivity, efficiency and specificity are shown. The proposed techniques are for rapid diagnosis of *D. destructor* nematodes. Designing specific primers for study populations could be a useful tool to help life scientists expand and continue their research.

**Basic provisions for defense:**

1. Morphometric variability of geographically distant populations of *D. destructor* from Russia and Iran was studied.

2. The molecular variability of *D. destructor* populations from Russia and Iran was studied.

3. New species-specific primers for molecular diagnostics of *D. destructor* have been developed.

Approval of the work's findings. At the international nematology conference, the research findings were presented and discussed (Nizhny Novgorod, 2018) and the scientific forum (Moscow, 2020).

Publications retrived from the outcomes of the dissertation research, 8 papers were published, including 2 articles in scientific journals indexed in Scopus, 3 articles in peer-reviewed scientific journals included in the list of BAK, and 3 abstracts from conferences.

**Personal contribution of the author.** The applicant participated in setting the goal and objectives of the study; collected and analyzed the material obtained, processed and interpreted the data, and prepared publications in co-authorship.

**The dissertation's structure and content.** The dissertation is 108 pages, subdivided into an introduction, three chapters, conclusions, and applications, and contains 20 tables and 24 figures. The list of references includes 123 sources in foreign languages and one in Russian.

**Acknowledgment.** The author expresses her deep gratitude to the supervisor Pakina Elena Nikolaevna, for her scientific ideas, help, and attention to the work. The author thanks M.V. Pridannikov for providing the potato samples from various regions of Russia Federation, as well as for their invaluable contribution to the revision of the Russian version of the dissertation. The author thanks the Organization for Agricultural Research, Education, and Extension of Iran (AREEO) for providing the potato samples from Iran.Finally, I owe thanks to a very special person, my husband, Yousef Naserzadeh for his continued and unfailing love, support and understanding during my pursuit of Ph. D degree that made the completion of the thesis possible.

# Chapter 1. LITERATURE REVIEW

## 1.1. Problematic of genus *Ditylenchus*

### 1.1.1. Overview of nematodes from the *Ditylenchus* genus

All types of agricultural plants can be affected by various parasitic nematodes, which lead to a decrease in yield and quality. The annual loss of crop yields due to pest nematodes is estimated at about 5% of the world's food production, worth US $125-157 billion [3, 24]. However, it points out that losses are much higher because many plant producers, mainly in developing countries, are not aware of the harmfulness of parasitic nematodes. Thus, the average yield loss of some crops can reach 20% per year [5, 25].

The *Ditylenchus* genus of rot nematode one of the most problematic genera of nematodes that parasitize plants. The outline of the species and the danger posed by individuals of this genus are the subject of numerous research works.

More than 90 known nematode species make up the genus *Ditylenchus*. This species has a wide range of food specializations and occupy a variety of ecological niches. The majority of *Ditylenchus* species are free-living nematodes that inhabit the soil and feed on fungi. Some are obligate parasites of higher plants [26, 27]. Stem nematodes have a variety of trophic preferences and other biological niches; in which they are comparable in this respect with the genus *Aphelenchoides* [28].

The potato nematode (*D. destructor*) and stem nematode (*D. dipsaci*) cause a variety of symptoms depending on the host species and the type of tissue affected. At the same time, all *Ditylenchus* species that parasitize plants are very similar to each other and have very few morphological differences [29, 30].

There are many poorly documented species in this genus, and some species from other genera can be taxonomically assigned to the genus *Ditylenchus*. The classification within this species of nematodes are challenging because of the lack of isolated features and the physical similarities of numerous species. The wide geographic distribution of *ditylenchus* species can be understood as the origin of the evolution of many species of this genus [10, 31].

The taxonomic position of the genus *Ditylenchus* in the order *Tylenchida* has changed several times. Now it is the subfamily *Anguininae*, the family *Anguinindae*, the superfamily *Tylenchoidea*, and the phylum Nematoda. The number of species in the genus *Ditylenchus* is constantly changing. Some species have been transferred to other genera of the order *Tylenchida* [32].

Of the only three species of this genus, *D. destructor*, *D. dipsaci*, and *D. angustus*, are crucial to the economy as parasites of various crops and ornamental plants. *D. destructor* is also able to feed on fungal mycelium [33]. Some species of this genus have a very wide range of different hosts, but no reports of pathogenicity have been reported for them [34].

### 1.1.2. Phytoparasitic Phytoparasitic species belonging to the genus *Ditylenchus*

The most important and economically significant species include *Ditylenchus destructor* stem (or tuber) potato nematode (potato tuber rot nematode), *Ditylenchus dipsaci* (bulb-stem nematode), *Ditylenchus angustus* (stem rice nematode), *Ditylenchus gigas* (stem bean nematode) and *Ditylenchus africanus* with *Ditylenchus arachis* (peanut pod nematodes) [35, 7, 12-17].

In the Eurasian region, *Ditylenchus dipsaci* and *Ditylenchus destructor* are two of the most significant plant parasitic nematode species. Although *Ditylenchus dipsaci and Ditylenchus destructor* are quite similar, their pathophysiology is different [17, 35].

*Ditylenchus dipsaci* has extensive intraspecific variability, which includes more than 20 biological races with different host ranges, as well as the presence of different degrees of isolation in reproduction and speciation [6, 18, 36].

## 1.2. A description of the *Ditylenchus destructor* species

### 1.2.1. History of the study of *Ditylenchus destructor*

Nematode that causes potato tuber rot was first identified by Kuhn and was described by Anguillula *dipsaci* [37]. Then in 1936, Filipiev synonymized it as *D. dipsaci* [19]. But Thorne, having shown the morphological differences of this species from *D. dipsaci*, singled it out as an independent species, *D. destructor* [20]. Before A

new species called *D. destructor* has been reported [4, 38], it had long been considered a race of *D. dipsaci*. Thus, most of the old works contain basic information about these two species, especially about their harmfulness to potatoes [21, 39].

The genus *Ditylenchus* includes 67 species according to [10, 40] and 80 species according to [41].

A key to identify *D. dipsaci* and *D. destructor* has been developed [42]. However, the identification of species within a genus is sometimes problematic due to very small differences between species.

Domain: *Eukaryota*

Kingdom: *Metazoa*

Type: *Nematoda*

Class: *Secernentea*

Order: *Tylenchida*

Family: *Anguinidae*

Genus: *Ditylenchus*

Species: *Ditylenchus destructor*

Adult *D. destructor* show significant morphometric variation according to their host and age. Males and females are similar in appearance. Lateral field with six incisures, the number of which decreases to two in the area of head and tail. *D. destructor* is similar to *D. dipsaci* but has six lateral field incisures (as opposed to four), a longer postvulval sac, and a rounded caudal end [1, 23, 43].

*D. destructor* lives in the underground parts of plants, stolons, roots, rhizomes, and tubers of plants. Potatoes are among the main hosts for this parasite. The potato (*Solanum tuberosum* L.) plays a very important role in global food security, and one of the most significant pests for this crop is *D. destructor*.

### 1.2.2. *Ditylenchus destructor* distribution and host plants

The wide geographic range of *Ditylenchus* is explained by the evolution of the ancient origin of this taxonomic group [18, 21, 44]. However, many countries with an important agricultural sector have taken legislative action against the spread of *Ditylenchus* nematodes. According to [45], this trend is increasing, as the analysis data shows that *D. destructor* was included in the regulatory legislation in 12 countries in 1982 and already in 53 countries in 2000. The situation is similar for *D. dipsaci* in 23 and 46 countries, respectively. Hockland and colleagues [46] suggest that this is for the reason that these species are easily transferred in the international plant trade due to their endoparasitic nature.

The ProMED (Electronic Emerging Infectious Disease Outbreak Reporting) plant disease reports published between 1996 and 2002 indicate that nematodes are only minor pathogens, causing about 1% of emerging plant infectious diseases [47]. This was assumed to reflect the proportion of targeted research interests rather than the actual proportion established by the biodiversity inventory. According to all this, it was observed that *Ditylenchus* species are not frequently mentioned in literature [3, 48].

However, there is little data on the allocation of funds for potato stem nematode control. After species differentiation in 1945, *D. destructor* was registered in many countries, mainly in temperate regions [16, 49]. According to [12, 50], the potato stem nematode is present in more than 70% of the member countries of the organization in European territories. Most countries indicate that the pest is of limited distribution or that there are only a few published data points on the occurrence of the species. *D. destructor* sightings have been reported everywhere in the globe, including the United States, Canada, Peru, most European countries, Russia, Iran, Pakistan, Bangladesh, China, Japan, Hawaii, New Zealand, and South Africa [3, 28, 29, 51].

Despite a general tendency to be harmful in colder and wetter regions [30], *D. destructor* was discovered in Iran's arid or semi-arid provinces of Semnan and Tehran,

where potato cultivation relies heavily on irrigation. The percentage of infection with various nematodes causing various root lesions was 87% and 54% in Semnan and Tehran, respectively [31, 52]. The percentage of *D. destructor* field infestation was 11% in Semnan and 2% in Tehran samples [31, 53].

*D. destructor* on potato potatoes has been recorded in Iran from various regions with different climates, including Karaj, northern cities, Tehran, Semnan, Hamadan, Fars, West Azerbaijan, Ardabil, Isfahan and Chahar Mahal and Bakhtiari [31-33, 54].

Similarly, to *Ditylenchus* sp. was found in 28% of samples from six major potato producing regions in Saudi Arabia in a survey conducted in 1989-1991 [34, 55].

*D. destructor* has been described on potatoes and carrots [35, 56], potatoes in Peru [36], ponderosa pine in California, USA [57], and Cimicifuga racemosa from Norway [37, 58]. In addition, the species is found on cabbage, carrots, beets, onions and other agricultural crops in Poland, on ornamental plants (hyacinth, crocus) and some weeds.

This species was noted for strawberries in Moldova [38, 59]; on potatoes in Lithuania [38]; on flowering plants [39, 60] and potatoes [40] in Ukraine; in Bulgaria on hops (*Humulus lupulus*) [41]; on potatoes, onions, lupine and other crops in Belarus [42, 61, 62].

In Japan, *D. destructor* damages 18 host plants including Brassica chinensis, *B. oleracea*, *Capsicum annuum*, *Dendranthema morifolium* (*Chrysanthemum morifolium*), *Cucumis sativus*, *Cucurbita moschata*, and *Lycopersicon esculentum*. However, serious problems arise mainly with iris and garlic, and foci of infection continue to spread despite control measures [43, 44, 63].

In Korea [45, 64], it was found to parasitize sweet potatoes, potatoes, mint, and ginseng. This nematode was first reported in New Zealand as a hop pest in the South Islands [36, 65].

In China, it was first found parasitic on sweet potatoes, potatoes, and mint [46]. From 2004 to 2006, potato rot was observed on American ginseng (*Panax quinquefolium*) grown in the Beijing area and Hebei province in China [25, 66]. Pathogenicity tests confirmed that *D. destructor* caused the disease [48].

*D. destructor* has been reported in the USA on potatoes in Idaho [67, 68] and on pine (*Pinus ponderosa*) in California, USA [36, 69].

Since the *D. destructor* e was removed off the EPPO quarantine pest list in 1984 as a result of its extremely widespread distribution in the area, the general quarantine regulations for it have been lifted in all European territories [70].

Currently, *D. destructor* is subject to regulation under the Plant Health Directive of the EU. For instance, it is listed as a pest whose introduction and distribution should be prohibited in all member states if present on corms of Crocus and flower bulbs, Hyacinthus, Iris, Trigridia, Tulipa, miniature cultivars, and tubers of potatoes meant for growing (Figure 1).

Figure 1. - *Ditylenchus destructor* distribution map in the world (www.CABI.org) [21]

The species *D. destructor* is a polyphagous nematode and has been identified as hosting 90 plant species from different families.

[50] states that about 70 crops and weeds, as well as the same number of fungal species, have been recorded as hosts of this nematode [51, 71].

The *Destructor* feeds on ornamental plants (hyacinth, crocus) and some weeds. The main significant hosts for *D. destructor* are cabbages, sweet potatoes, and bulbs, and pattern tulips, gladiolus, and dahlias are good hosts.

From root crops, it affects sugar beets, chards (*Beta vulgaris),* and carrots (*Daucus carota*). Other common hosts include sunflower, onion, garlic, peanut, tobacco, sugar beet, cucumber, tomato, sugar cane, carrot, and alfalfa. As noted in [52, 72], *Trifolium pratense*, *T. repens*, and *T. hybridum* are good hosts for *D. destructor*; *Festuca pratensis* and *Medicago sativa* are less good hosts.

### 1.2.3. Distribution and harmfulness of *Ditylenchus destructor* in Russia and Iran

The peak of potato nematode rot in the former Soviet Union occurred in the 1960s–70s [41, 73]. In Russia, *D. destructor* was found on 11 out of 13 weed species commonly grown in potato fields near Moscow [4, 74].

*Solanum nigrum*, *Taraxacum officinale* (20%), and Barbarea vulgaris (16%) were the most heavily infected; Fumaria officinale and Matricaria inodora were also hosts of this nematode [39, 75].

In Belarus, *D. destructor* is unevenly distributed and occurs mainly in the Minsk region [53]. In the Minsk region, 69% of all studied batches of seed potatoes turned out

to be infected, while in the Brest region this figure was 26; in the Gomel region 29; Mogilev 30 and Vitebsk 33% (Figure 2).

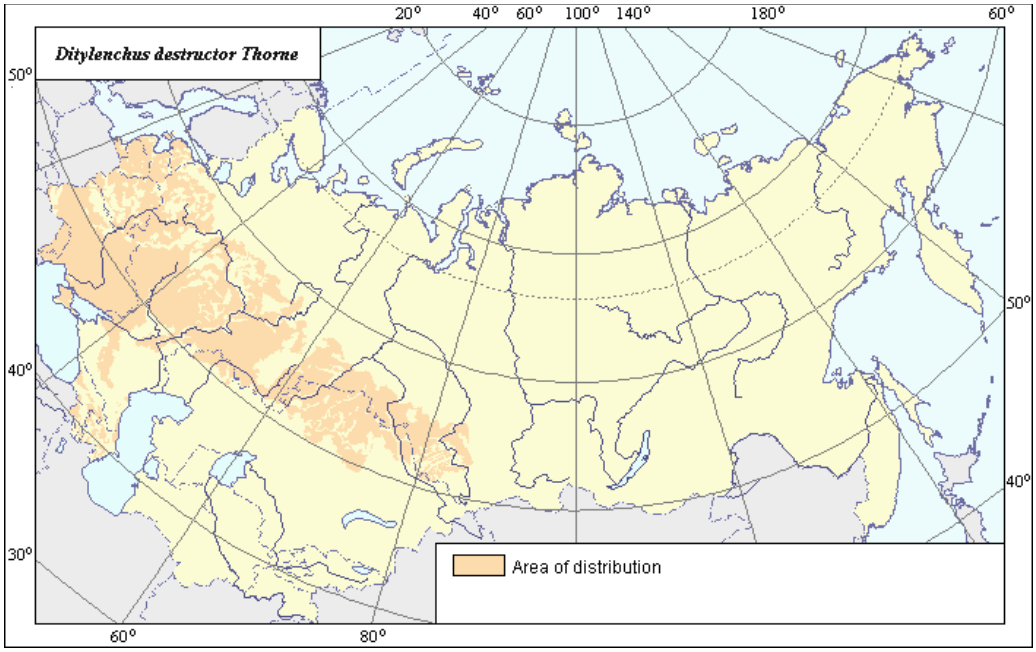



Figure 2. - Distribution of *Ditylenchus destructor* in the former USSR (www.agroatlas.ru) [54]

It is reported that studies of *D. destructor* in Iran are carried out only in selected regions, and the role and significance of such studies have not been studied. In Iran, *D. destructor* was first discovered and identified on potatoes in 1971 in the Karaj region and some northern parts of the country [55, 76]. Schorfe and Heyri studied potato tuber rot caused by nematodes in Fars province and observed its high population of infected tubers [56]. Barooti pointed to *D. destructor* in Ardebil province as being among the most crucial nematode species in that province [57]. In a study [31] on native parasitic nematodes and their population density in potato fields in the provinces of Tehran, Semnan, [77, 78] and West Azerbaijan, a high degree of damage by these nematodes in potato fields and warehouses was noted in Tehran and the province of Semnan [31]. Giti and colleagues identified this species while studying plant-parasitic nematodes in the potato fields of the city of Hamadan [56, 79]. In a study conducted [33], in addition to other types of nematodes found in the soils of the provinces of Tehran and Markazi, *D. destructor* was also found. In Iran, a report of *D. destructor* has been made to be

associated with many crops such as potatoes, wheat, beans, soybeans, eggplant, tomatoes, tea, corn, oranges, and alfalfa.

The tuber nematode is a serious potato pest in the range of 15-20 °C and relative humidity above 90% [6, 21, 80]. However, according to [54], the tuberous nematode in Belarus is favored by hot, dry weather and moisture deficiency in the soil. Similarly, *D. destructor* was reported in 1993 to be present in both cold and warm regions of Albania with an infestation rate of 3.5–12% [58–60].

The damage threshold for *D. destructor* is 20-50 individuals per 1 kg of soil. Even in heavily infested fields, populations of this nematode can be eradicated or reduced if the absence of biennial or perennial host plants is observed in the field for several years [53]. Weeds, on the other hand, may play a beneficial role in modern agriculture and horticulture by providing diversity, ecosystem functions, and support for many other species [61].

### 1.2.4. Biology and parasitism of *Ditylenchus destructor*

*D. destructor* infects the underground organs of plants and is rarely found in the above-ground parts. This nematode is an interstitial parasite that migrates underground to plants, where it grows and continues to live, destroying and rotting plant organs such as potato tubers, lily bulbs and garlic. This nematode enters potatoes through the stolon and lentils. The nematode continues to grow even after tubers are harvested [21].

[51] pointed out that the potato rot nematode, although superficially similar to the onion stem nematode (*D. dipsaci*), is not capable of forming nematode "tangles" and cannot easily withstand drought. However, it can persist in the soil on weeds and host fungi. Optimal temperatures for nematode hatching at 28°C are given, but different species are adapted to different climatic conditions. For example, in Europe, the temperature required for hatching was lower [62]. De Weil et al. [63]. Conducted experiments with populations of *D. destructor* in South Africa and found that the nematode produces callus tissue in various potato varieties, including BP and Bufel.

This nematode can only move a short distance in the soil. Transmission through contaminated soil is another important route of spread. Nematodes additionally found in irrigation water. *D. destructor*. And because of this, this species is only important in well-moistened soils.

These nematodes can also overwinter in the egg stage. Thorne's research shows that *D. destructor* hibernates on American farms in the soil as eggs and adults. According to Annon's study, in Ireland, nematode survival in the soil continued in the presence of mint residue and unharvested potato tubers. Larvae immediately after hatching in spring are able to parasitize hosts [21].

The nematode can develop and multiply at a temperature of 5-34°C, with an optimum at 20-27°C. The development of the generation of nematodes takes 18 days at 27-28 °C. At 20-24 °C - from 20 to 26 days, and at 6-10 °C lasts 68 days. In Alma-Ata region (Kazakhstan) in the former Soviet Union, 6-9 generations of potatoes for this nematode were recorded [8, 81]. Most nematode infestations occur at 90-100% relative humidity, but they cannot survive at less than 40% relative humidity [82].

The *D. destructor* is adapted to various climatic conditions and can cause damage to plants in the temperature range from 5°C to 30°C [64]. However, studies in South Africa have shown that the hatching process starts at 28°C and results in larvae in about 5 days. The development of nematodes from larvae to full maturity is also achieved in 6–7 days [62, 83].

Nematode-infected tubers and soil containing *D. destructor* are two important sources of soil contamination of potato tubers. Rich [65] reported that the onset of infection was in 70% of the tubers in the area of the stolon junction, 18.8% in the area adjacent to the stolon junction; 5.1% of tubers were infected over their entire surface, and in 6.1% of tubers infection was observed only at the top.

Once the tubers germinate, they migrate to the underground stems and rhizosphere [84]. Then, until the flowering stage, the nematodes can be outside the shoots to a limited extent. Then they begin to migrate to stolons and young tubers, and

that is when these organs are formed. First of all, young tubers around the stolon are affected and infected [67].

The nematode causes cell necrosis and cavities in the potato tuber. Thus, in the early stages of infection, the dots become white, which become discolored and therefore differ from the surrounding healthy tissue. The periderm layer under the skin is actively affected by the nematode, so the cavities and cells surrounding the nematode feeding site can easily be separated from the healthy tissue surrounding them [85]. The small cavities gradually become larger and darker, and form a mass of these cavities. Then necrotic spots of the main tissue of the tuber gradually appear. The fabric contains many pigment granules. At this stage, the cell dies, leaving only the remnants of the cell walls [67].

In potatoes at harvest, the first symptoms appear as gray to white gypsum spots that become visible after the skin is removed [86]. Usually spots are formed after the ingress of saprophytic bacteria and fungi. In this case, the skin of the potato tubers shrinks, and in case of severe damage, the entire surface of the potato tuber rots and is crushed. Such tubers have no commercial value. The nematode continues to develop in warehouses, in addition to decomposing infected tubers, switching to feeding on other healthy tubers [56] (Figure 3).

Figure 3. - Potato tubers infected with *Ditylenchus destructor*

Damage to carrots is expressed as longitudinal lines on the crop's root surface and white areas in the subcutaneous tissue. These fragments are clearly visible in the cross section. Seeds and small seeds usually show no visible symptoms, but larger seeds, such as beans and pods, may have yellowing skins and colorless spots [56, 87, 88].

In onion flowers and stems, infection commonly starts at the onion's root and progresses to fleshy scales with yellow or dark brown welts. Secondary rot can occur and the plant dies [89]. Individuals of *D. destructor* accumulate at the border of the affected and healthy parts, but rarely separate from the destroyed tissues [68].

### 1.2.5. Morphology and taxonomy of *Ditylenchus destructor*

The morphology of *D. destructor* is described in [20, 28, 51, 58] and [69]. [72, 90] provides more detailed information on the morphology of the reproductive system and esophageal glands of *D. destructor*.

Females: length = 0.81-1.4 mm; a = 30-35 μm; b = 8-10 μm; c = 15-20 μm; V = 78-83%.

The general shape of the body is worm-like; after fixation, it is slightly curved towards the abdomen. The shape of the end of the body varies from person to person and is rarely seen in the annular state. The pores of the amphids are visible as small dots on the lateral lips. The cuticle of the head hardens. The short stylet has rounded pointed knots at the ends, the anterior surfaces of which are turned backwards. The length of the stylet cone is between 45% and 50%.

It has an average muscular bladder about 3 microns thick in the duct. On the side surfaces in the middle of the case there are six side field lines that can be cut to two ends. The secretory pore is opposite the esophagus [24].

Males: length = 0.8-1.3 mm; a = 34-40 μm; b = 7-8 μm; c = 12-16 μm; T = 73-80%. Males are very similar to females and differ from them in the structure of the pelvic organs. The male reproductive tube has a long testicle that can reach up to the esophagus. Spermatocytes are arranged in two or more rows. The length of the spicules varies from 24 to 27 μm. The bursa covers 50-90 percent of the length of the tail. The

tail is equal to that of females or slightly narrower, with a very rounded tip [24, 91] (Figure 4).

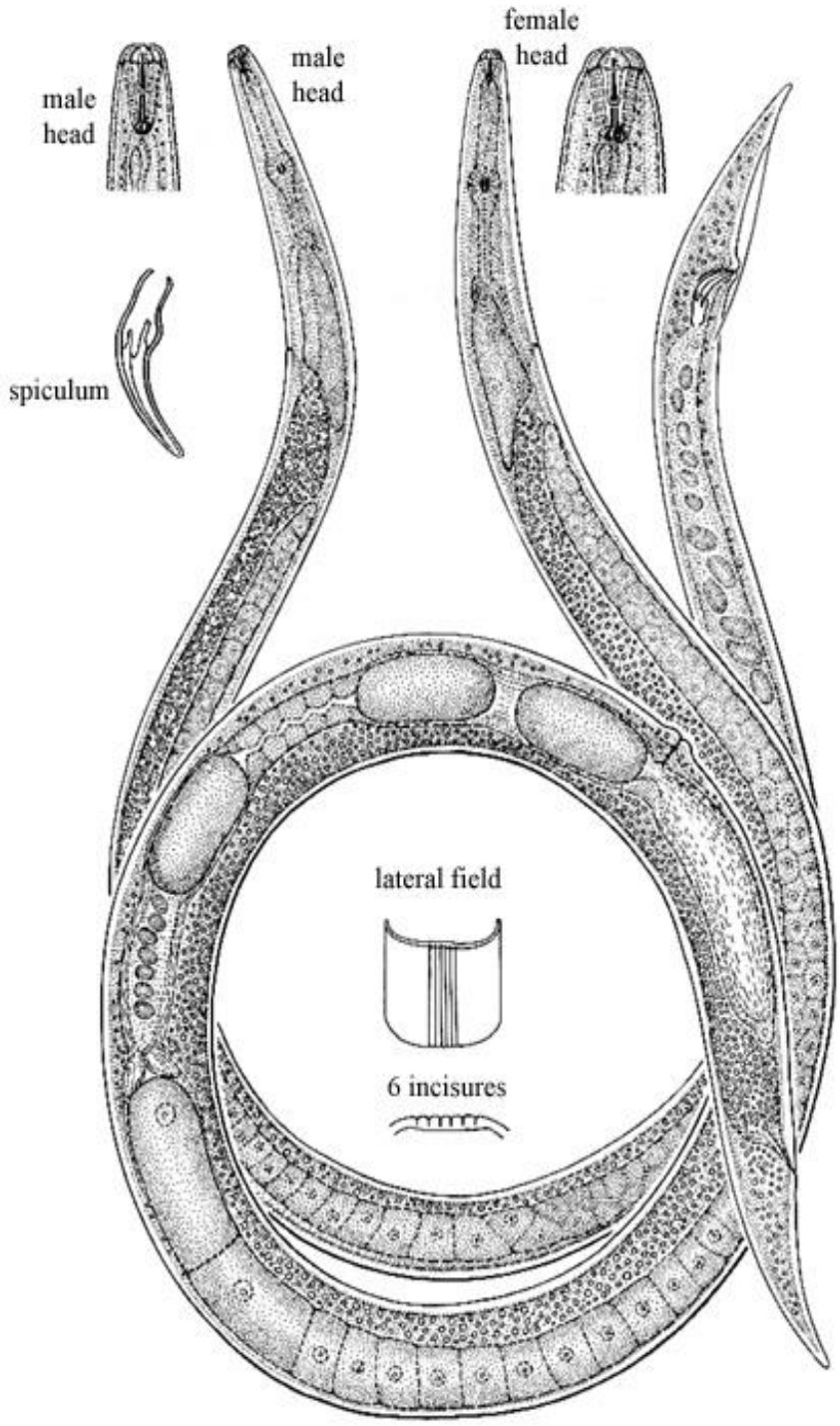



Figure 4. - *Ditylenchus destructor* (male and female) [69]

*D. destructor* is very similar to *D. dipsaci*. According to Brzeski's key, all morphological characteristics of the two species, including factors C and, bursa length, and stylet length, are the same. The most noticeable difference between the two species is how many lines there are in the surface's lateral field, which is six in *D. destructor* and four in *D. dipsaci*. Of course, there is another difference. For example, a variation of the tail in *D. destructor*. As was said, the tail end is rounded, while in *D. dipsaci*, like in the species *D. convallariae*, it is pointed.

The two species, *D. destructor* and *D. convallariae*, although very similar, have slight differences. The differences between the two species are due to the thickness of the esophageal tube, the number of lateral incisura, the stylet length, placement of the vulva, as well as similar factors C and the length of the bursa. But the tail end of *D. destructor* is rounded, while that of *D. convallariae* is elongated (the tail ends at one point). In addition, the length of the Pus is about 53–90 μm in *D. destructor* and about 27–47 μm in *D. convallariae*.

As a rule, species identification is difficult due to significant interspecies differences in morphological characters, which often depend on the size or age of the nematode [51, 91].

Potato rot nematodes are active in the underground parts of the plant (roots, stolons, and tubers); *D. dipsaci* in leaves, flowers, and stems, and *D. convallariae* on fungal mycelium. In addition, the length of the Pus to the distance of the vulva-anus in this species is greater than in *D. myceliophagus* [73]. *D. destructor* is similar to *D. arachis* [14, 92], *D. afronus* [74], *D. halictus* [75], *D. longicauda* [76] and *D. oncogenus* [77], but none of them is pathogenic for potatoes [93].

In addition, *D. destructor* can be distinguished from *D. arachis* and *D. africanus* with its comparatively long spicules and distinct host preferences, from *D. convallariae* with a distinctly shaped tail tip, and from *D. halictus* with its relatively longer body, comparatively larger spicules, and a distinct reproductive strategy (sexual process or parthenogenetically).

It differs from *D. longicauda* with relatively large spicules, a relatively higher PUS/Vulva ratio and a distinct tail shape (dense with a rounded tip compared to long and thin, pointed to a rounded tip). It differs distinctly from *D. oncogenus* in the shape of the tail tip.

For comparison, the two species *D. destructor* and *D. dryadis* meet almost all the analysis criteria in the key [78], and only in *D. destructor* the stylet length is slightly (by about 2 microns) longer (the stylet length is about 10-13 microns in *D. destructor* and about 10-11 microns in *D. dryadis*). In addition, the caudal end of *D. destructor* is round, while that of *D. dryadis* is pointed [94].

### 1.2.6. Plant protection measures of *Ditylenchus destructor*

Due to the diverse variety of hosts and several generations every crop cycle, it is challenging to regulate the amount of *D. destructor* that is present in the field [51, 79]. Several weed species are hosts for this nematode, making crop rotation a limited option for reducing nematode numbers in the soil [95].

The use of nematicides to control nematodes is the most effective way to control the abundance and harmfulness of individual species. At present, the drug Vidat 5G (50 grams of oxamil) is registered in Russia in the form of granules for application to the soil when potatoes are planted. This drug has a biological efficiency of 90–98% and can effectively reduce damage to potatoes by stem nematodes. Nevertheless, the high cost and high toxicity to warm-blooded animals spurred the search for other control methods, even in intensive agriculture. Potato cyst nematodes can be effectively controlled via nematode tolerance and resistance, but there is currently no reliable information on the resistance of most commercial potato varieties to the stem nematode. Tests for the resistance of potato varieties to *D. destructor* were started in the early 1950s [51], but no significant results were achieved in this area. Since then, only a few research teams have carried out research in the field of breeding potato varieties for resistance to the potato stem nematode [96]. An approximate list of potato varieties, according to resistance to the stem nematode, is given below:

**Sustainable:** Temp, Yahant;

**Weakly affected:** Zhuravinka, Outflow, Synthesis, Talisman, Zarnitsa, Zdabytok, Climber, Padarunak, Orbit, Suzorye;

**Medium affected:** Veras, Koretta, Belorussky-3, Zhivitsa, Caprice, Colorit, Brigantine, Willow, Breeze, Kupalinka;

**Strongly affected:** Odysseus, Doe, Archidea, Lasunok, Dina, Garnet, Altair, Vetraz, Treasury, Blakit;

**Very strongly affected:** Milavitsa, Guslyar, Delikat, Garant, Atlas, Lapis Lazuli, Fresco, Adretta, Lugovskoy, Rosinka, Yavar, Sante, Nikita, Dolphin, Krinitsa, Neptune, Dar, Zhivitsa (http://agrosbornik.ru) [54].

As new varieties enter the potato market each year, it is necessary to systematically test for resistance and tolerance in modern potato cultivars.

The lack of resistant and tolerant varieties in widespread potato production and the need to minimize the use of nematicides have sparked research interest in alternative management strategies, such as the use of antagonistic organisms.

Numerous fungal and bacterial antagonists have been investigated in the management of various nematode species [80, 97]. However, fungal antagonists have not been very successful in controlling the abundance and severity of *D. destructor*, partly because this nematode feeds on fungi [81]. *Beauveria bassiana*, an entomopathogenic fungus, has been successfully integrated into the management of the Colorado potato beetle and is therefore closely related to the potato plant as an entophyte [81, 82].

The spores of these fungi can survive in the soil after a single application and be effective against overwintered Colorado potato beetle larvae [83]. The testing of this fungus against the potato stem nematode showed its inefficiency in controlling the number of nematodes in the soil [83].

In an attempt to decrease the spread and thus reduce damage to crops as a result of infection by this nematode species, more than 50 countries in the world have introduced phytosanitary regulations in relation to trade in agricultural products, which is [84] the main route of distribution. Nematode's effect on trade, especially on seeds intended for planting, is enormous [85].

In the recent past, new cases of damage to garlic and sugar beet crops caused by *D. destructor* have been reported [86]. The interaction between the stem nematode, tuber rots and other pathogens in the potato plant remains relevant due to its phytosanitary significance and the potential high level of potato damage by this nematode species. Understanding these interactions and the factors that influence them is vital to developing management strategies.

### 1.2.7. Management of *D. destructor*

Due to the vast host range and many generations each vegetative cycle of host crops, managing *D. destructor* once it can be found in the field is a challenging undertaking [1, 19, 98]. Crop rotation is a limited option because this nematode is a host to a number of weed species [1,5]. Even in intensive agriculture, the usage of nematicides to control nematodes has forced researchers to investigate alternative management methods [99].

It's been demonstrated that nematode resistance and tolerance are efficient methods for managing potato cyst nematodes, but there is no accurate information regarding whether types are resistant to potato rot nematodes. Early in the 1950s, tests for resistance in potato types against *D. destructor* were started [3, 12, 100]. Since then, trials for resistances have been discontinued.

It is necessary to assess the presence of resistance and tolerance in contemporary grown potato varieties because new varieties are introduced into cultivation every year. Research interest in alternate management measures, such as the use of antagonistic organisms, was sparked by the lack of resistant and tolerant cultivars and demand to reduce the use of nematicides.

Different nematode species have been managed using a variety of bacterial and fungal antagonists [88, 101]. However, because *D. destructor* is a nematode that feeds on fungi, utilizing a fungal antagonist to manage it hasn't been very effective [19, 102, 103].

As an entophyte, *Beauveria bassiana*, an entomopathogenic fungus, is strongly linked to the potato plant due to its excellent integration with the management of the Colorado potato beetle [104]. These fungi's spores can survive in soil after just one treatment and are useful for controlling Colorado beetle larva that overwinter there [105].

More than 50 nations around the world have implemented phytosanitary regulations on the trade of crop production, which are the main channels for dissemination, as a way to reduce the spread and resulting increased crop damage caused by infestation by this nematode species [50, 106]. Nematodes have a significant negative influence on trade, particularly with regard to seeds meant for planting [90,101].

Recent reports of *D. destructor* related crop damage to sugar beet and garlic have increased [4, 107]. Due to its significance for phytosanitary reasons and the potential for significant potato damage this species has on potatoes, the relationship between tuber rot nematodes, stem nematodes, and the potato plant is still relevant today. The creation of management strategies requires a thorough understanding of these relationships and the elements that affect them.

## 1.3. Molecular study of *Ditylenchus destructor*

### 1.3.1. Using PCR to diagnose Nematodes

The development of molecular-based detection tools for various groups of organisms has been widely adopted and successfully applied to the diagnosis of plant parasitic nematodes thanks to the invention of DNA sequencing, polymerase chain reaction (PCR), and the enormous amount of genetic data.

The following benefits of molecular detection instruments over alternative methods: They have the following advantages: 1) they can be used with high throughput; 2) it is simple to learn the structure of individual DNA segments using a variety of databases and sequencing data; 3) they are affordable, quick, and accurate; 4) DNA markers are not impacted by phenotypic variations or nematode developmental stage [108].

The accuracy of DNA-based markers enables precise classification and diagnosis. Since they can be used with a number of sample types, including eggs, adults, larvae, soil extracts, host tissues and fixed samples, they are simple to use, accurate, rapid, and versatile, DNA-based detection technologies are great for identifying nematodes [88, 109].

Much of the diagnosis of nematodes is based on PCR amplification of target DNA using primers for species-specific. The PCR technique has been developed as one of the methods most commonly used to study and classify the genetic diversity of nematodes. Differences in band structure between species or populations can be used as taxonomic markers [110].

PCR-based detection methods have revolutionized the field of nematode diagnostics and have been widely used due to their increased sensitivity, accuracy, speed, relative ease of operation, and cost-effectiveness compared to other diagnostic procedures [88-90].

One approach to developing DNA markers that can aid in the diagnosis of nematodes has generally been based on conserved regions of ribosomal DNA (rDNA), namely the outer transcribed spacer (ETS), and the inner transcribed spacers 1 and 2 (ITS1 and ITS2 respectively) [89, 111].

Thus, sequences that diverge between nematode species and are conserved in several isolates of the same species become an ideal target for the development of species-specific primers [112]. Ribosomal DNA regions were very suitable for target marker selection because they were multicopy genes and provided adequate diversity to be used for diagnostics and phylogenetic relationships between species [89].

Tandem repeats found in the rDNA sequences serve as easily detectable genetic markers that are used to construct phylogenetic trees [91, 92] and to evaluate and identify populations with similar genetic structure [93, 113]. Currently, the genus *Ditylenchus* has about 70 species [4, 10, 14, 94] and [75]. Due to the very similar morphology, the diagnosis of species within the genus *Ditylenchus* is a very difficult task [114].

Recent research using molecular techniques has shown that many alleged monospecific species actually belong to closely related or cryptic species, sharing common morphological diagnostic traits while being genetically diverse. This is important since earlier descriptions of nematode species have emphasized the concept of morphological or typological creatures [115].

To select effective schemes for the control of parasitic plant nematodes, it is necessary to accurately and quickly detect a specific nematode species. This is especially important for the purpose of identifying the species *D. destructor* due to the high degree of morphological similarity of characters in closely related species, as a result of which morphological identification is not always a reliable tool for the purpose of identifying the species *Ditylenchus* species [5, 97, 116].

Different sets of species-specific primers for *D. destructor* have now been created based on sequence differences between the rDNA-ITS region of this species and other *Ditylenchus* species sequences deposited with GenBank [5. 98].

Testing of diagnostic primers typically necessitates a greater quantity of samples with comparable morphological traits and a broad geographic dispersion [98].

The use of molecular methods for routine recognition is very effective for *D. destructor* populations from various host plants [61, 99–101], which used and recommended specific primers for *D. destructor* identification. It has also been reported that rDNA ITS regions are successfully used for phylogenetic analysis [32, 117].

However, the molecular characterization of the isolates can not be the only parameter to distinguish them at the species level. The unification of polyphasic taxonomy principles should serve as the foundation for this, integrating and incorporating all data and knowledge (phenotypic, genotypic, and phylogenetic) utilized to discriminate between taxa at all stages [77, 96, 104].

### 1.3.2. Molecular genetic identification of *Ditylenchus destructor*

The identification of nematodes from the *Ditylenchus* genus are challenging, as was already established. Because field populations of *D. destructor* exhibit significant variability, precise identification and detection of this species is crucial. Nematode taxonomists must therefore increasingly use a variety of techniques to identify these nematodes. When nematodes are identified using various molecular methods, as opposed to morphometric data, the research species can be identified accurately and quickly.

For *D. destructor* and *D. dipsaci*, the European and Mediterranean Organization for Plant Protection (EPPO) offers a diagnostic technique [1-105]. The policy encourages the use of both morphometric and molecular techniques for nematode species identification. The use of data gathered using these techniques is crucial for managing and evaluating the danger that these nematode species pose to human health.

PCR is characterized by three factors: selectivity, sensitivity, and speed [106, 118]. A wide range of DNA-based methods for detecting genetic variation is available and can be used or adapted for taxonomic and diagnostic purposes [107–112]. Amplification of specific genomic regions is a highly efficient way to detect inter-and intraspecific variation between genera and species, as well as within species [119].

Ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) are the two most common repeat regions for taxonomic and diagnostic purposes. Since polymerase chain reaction (PCR) technology is sensitive enough to analyze differences between closely related species or genera, [97] used polymorphism in restriction fragment length (RFLP) to separate *D. destructor* from *D. africanus*. PCR amplification of transcribed spacers within the genome (ITS 1 and ITS 2) combined with restriction enzyme analysis (PCR-RFLP) is a powerful tool for diagnosing nematodes but is more time consuming than the other two methods, such as Real-time PCR and species-specific PCR.

PCR for specific- species is the most accessible and time-consuming molecular method in the laboratory and different primers can be used depending on the specific DNA region and plant parasites. [13, 120] Used a real-time PCR test to describe differences between *D. dipsaci* and *D. gigas*.

This is the official nematode diagnostic tool but is not often used to identify *D. destructor*. Molecular diagnostics is becoming increasingly important, especially the study of the ITS-rDNA region, which is a much conserved taxonomic marker for many genera of nematodes. [113, 114] were the first to sequence the ITS1 region of *D. dipsaci*, but the GenBank database now contains more than 50 sequences of *D. destructor* derived rRNA fragments collected from various locations and host plants.

### 1.3.3. DNA sequencing

In the 1950s, researchers were still working to determine the first 3-D protein structures, but other biologists had already gathered a ton of indirect information. It was made up of a double helix-shaped, long chain resembling a molecule, with two of the four nucleotides forming each link.

The tiny DNA sequence alphabet (4 nucleotides as opposed to 20 amino acids) allowed for much easier and quicker reading as well as rapid automation, which led to a

revolution in the 1970s. Currently, the global rate of DNA sequence determination is orders of magnitude quicker than the rate of protein sequencing [115].

The chain termination approach (Sanger dideoxy), which is more frequently employed, and the chemical degradation process (Maxam-Gilbert) have both been developed for DNA sequencing [116]. As with PCR, chain-termination sequencing involves the synthesis of fresh DNA strands to complete a single-stranded template. Template DNA, DNA polymerase with reaction buffer, one primary, and a mixture of all four labels of deoxynucleotide (dNTP) and four dideoxynucleotides (ddNTP), each with a different colored fluorescent light, are the components of the sequencing procedure. Chain elongation continues as long as all four deoxynucleotides are present before a ddNTP is accidentally added into DNA polymerase [115, 117].

As a result, a variety of novel chains in various lengths is produced. A laser that produces a certain color of light excites the fluorescent molecule as each labeled fragment sinks to the bottom of the gel. A detector captures each band's fluorescence color. A computer program may represent a file series [118].

### 1.3.4. Analysis of DNA sequences

The four nucleotides that make up DNA have different bodies, but both have the same pair of hooks: 5' phosphoryl and 3' hydroxyl (pronounced five prime and three prime), referring to their positions in the deoxyribose sugar molecule, which is a component of the nucleotide chain. This is similar to the twenty amino acids that make up proteins. The 5' and 3' sites of the component nucleotides are then joined to create the DNA molecule.

The resultant DNA strand has an unused phosphoryl group (P) at the 5' end and an unused hydroxyl group (OH) at the 3' end after the nucleotides have been joined. The 5'-terminus and 3'-terminus of the DNA strand are respectively called these extremities.

The succession of a DNA molecule's constituent nucleotides described from the 5' to 3' terminus is known as a DNA sequence [119].

### 1.3.5. Returning the Sequences of DNA

Protein sequences are straightforward structures with a limited size range, clearly defined boundaries, and fundamental functional characteristics. Additionally, higher eukaryotes like those found in animals and plants or microbial proteins share many of the same characteristics. As one may expect, higher animals' equivalent gene sequences (DNA) are evolving to become more diverse and complicated. Human genes can be as short as a few thousand base pairs (bp) or as long as several hundred thousand bp. [120].

As one may expect, higher animals' equivalent gene sequences (DNA) are evolving to become more diverse and complicated. Human genes can be as short as a few thousand base pairs (bp) or as long as several hundred thousand bp. In eukaryotes (yeasts, plants, and animals), the protein-coding region is broken up into a variable number of segments of exons genes that contribute to the final protein and segments of genes that do not, called introns [121].

### 1.3.6. Sequence alignment value

The first step of any phylogenetic analysis is the alignment or analysis of positional homology between nucleotides or amino acid bases between closely related species that are descended from a common ancestor. Errors made at this stage can lead to misinterpretation of the data. Molecular Taxonomy and Phylogeny- The best way to compare homologous residues is to place sequences one on top of the other in a visual display, so that each homologous baseline is preferably in the same column of different sequences. There are three types of matching pairs: 1) matches (the same nucleotide appears for all sequences); 2) mismatches (different nucleotides were present in the

same position); and 3) differences (at least for one sequence, no base in a particular position).

Distance means that a deletion occurred in one sequence and an insertion occurred in another. However, the alignment itself does not distinguish between these mutational events. Optimal alignment of sequences of the same length can easily be done manually for closely related species. In distantly related species that have had several deletions or insertional mutations, alignments are usually built using computer programs with different algorithms. It is known that the optimal automatic alignment is one in which the number of variations and differences in accordance with the desired criteria is minimized. Clustal software, which uses a progressive alignment approach, is among the most widely used computer alignment programs. The sequences are ordered in pairs to create a space matrix, which is then used to recreate the phylogenetic tree.

This tree indicates the order in which gradual leveling should occur. Progressive alignment is a completely independent mathematical method of biological reality. The use of the structural components of this molecule will significantly improve the homology estimates, thereby providing a better alignment [103, 115, 122].

### 1.3.7. Phylogeny and genetic classification of *Ditylenchus*

The most useful criterion for classifying species is their phylogenetic relationship, namely a common origin, since such a relationship usually conveys the most detailed information about the characteristics of a taxon member. A taxon must be a monophyletic group descended from a single common ancestor, as opposed to a paraphyletic taxon, which includes only a few descendants of a common ancestor, or a polyphyletic taxon, whose members share only a distant common ancestor and are usually limited in other characteristics (e.g., homoplasy). Numerical taxonomy concepts were implemented in the 1950s based not on a few important features but on multi-character data. Numerical analytical methods were used to construct diagrams of the

general similarity of organisms. Such a diagram, called a phenogram, was supposed to give the description an objective basis.

This approach does not take into account the consequences of parallel or convergent evolution in taxonomic interpretations. The argument that a classification would strictly reflect only phylogenetic relationships was based on a different method than the degree of adaptive divergence or general similarity. Classifications based on phylogenetic principles are called phylogenetic classifications; only common, distinctly similar states of characters testify to phylogenetic relationships.

The method of phylogenetic inference is known as cladistics. Branching diagrams constructed using cladistic methods are sometimes called cladograms, and monophyletic groups are called clades [115, 123]. The maximum likelihood method for interpreting phylogenetic trees is an approach that is generally considered to maximize the use of data to obtain the most reliable estimates of phylogeny. it is the probability that the tree gave rise to the collected data. The main idea of the method is to calculate the probability of the observed data, assuming that they arose within a certain evolutionary tree and a given probabilistic replacement model.

### 1.3.8. Maximum likelihood method for interpretation

ML is an approach that is generally considered to use the data as much as possible to obtain the most reliable phylogeny estimates. The main idea of the method is to calculate the probability of the observed data, assuming that they have developed within a certain evolutionary tree and a given model.

The tree is probably the most accurate representation of the real phylogeny. The main problem with the widespread use of machine learning methods is related to computation time, because algorithms that find a machine learning score must search for a tree in a multidimensional parameter space. A sequence evolution model, a tree, and observed data are all components of machine learning [103].

# Chapter 2. MATERIALS AND METHODS

## 2.1. Materials

### 2.1.1. Collection of plant material

Seven samples of potatoes from two nations were examined to determine populations of *Ditylenchus destructor*. Mid-September in Russia saw the direct selection of potato material from farms in the regions of Moscow (Gala), Nizhny Novgorod (Colombo), and Bryansk (Innovator) regions (Figure 5).

The Agricultural Research, Education and Extension Organization (AREEO) in the middle of July in Iran took samples of tubers from farm areas in the provinces of Hamadan, Isfahan, Zanjan, and Ardebil (Satin, Santa) (Figure 6).

The potato plants were visually inspected during the growing season. The timing of harvesting is related to the cultivation technology in the regions, and the symptoms of *Ditylenchus* appear precisely during the ripening of tubers before harvesting.

In all seven regions, potatoes were plucked at random from various field rows. Therefore, excised potato tubers were visually inspected, and if any physical symptoms were noticed, they were collected (Figure 7).

Healthy tubers were sometimes collected for additional research. After digging up the tubers, they were washed and examined for the presence of external signs of the the disease.

*Ditylenchus*-symptomatic tubers were labeled and put in plastic bags. The tubers were lined with filter paper and kept at +4 °C for further research.

Figure 5. - Regions of collection of infected potatoes in the Russian Federation

Figure 6. - Regions of collection of infected potatoes in Iran

Figure 7. - Collection and inspection of potato tubers in the field

## 2.2. Methods of morphological analysis of *Ditylenchus destructor*

### 2.2.1. Isolation of nematodes from plant material

The peel was removed from potato tubers with symptoms of *Ditylenchus* damage in places of darkening of the tissue. Next, small sections of plant tissue were cut off and placed in small petri dishes; they were filled with tap water and left for half an hour at room temperature. The nematodes emerge from the plant tissue and sink to the bottom of the cup. After that, petri dishes were viewed under a stereomicroscope for the purpose of nematode detection.

Nematodes were individually selected with a special needle and transferred to a new petri dish with pure distilled water.

After that, the nematodes were transferred to a drop of distilled water on a slide of glass (Braunschweig, Menzel GmbH, Germany) and covered with a coverslip. In this

case, the coverslip is placed at an angle to the slide, above a drop of water with nematodes.

The coverslip was fixed to the slide with nail polish, and the slide was then gently heated on a thermal table to 80–85°C or over an open flame for a few seconds to kill and fix the nematodes and minimize the quantity of water bubbles.

Nematodes for preliminary identification were studied under a microscope with ZEISS Axioskop Imager A1 (Germany) at 10x magnification.

### 2.2.2. Morphological analysis of *Ditylenchus destructor* populations

To perform morphometric measurements, 200 male and female nematodes were manually selected for each population from Iran and 50 nematodes from the Russian Federation, and temporary slides were made.

Using AxioVision® software version 4.8.2, On a computer screen, light microscopic images and morphometric data were taken from digital photos (Carl Zeiss).

Nematodes were measured on a ZEISS Axioskop Imager A1 microscope at 40x magnification. Six morphometric parameters were used: body length and width, stylet length, postvulvar sac length (PUS), and vulva-anus distance in females, and spicule and bursa length in males.

Morphometric measurements are converted to micrometers (m) (unless otherwise indicated) and were obtained at various magnifications based on the relevant attribute.

In the analysis of morphometric data, if appropriate, references to *D. destructor's* initial description were made [20, 51], for the population from Russia [94], and for populations from Iran [11].

## 2.3. Molecular analysis methods

### 2.3.1. Extraction and purification of DNA

For molecular measurements, 50 nematodes from each population were used. All procedures were performed according to the VNIIKR standard or other standards using a reagent kit (EPPO Protocol, 2017) [105] (Table 1).

Table 1. - Chemical components of DNA extraction

| № | Name | Description |
|---|---|---|
| 1 | Proteinase K | Reagent for cell lysis, 1.0 µl |
| 2 | Lysis solution 2 | Reagent for cell lysis, 31 µl |
| 3 | Precipitating solution 1 | Protein Precipitation Reagent, 11 µl |
| 4 | Precipitating solution 2 | DNA Precipitation Reagent, 31 µl |
| 5 | Wash solution | DNA Wash Reagent, 41 µl |
| 6 | DNA solution | DNA Dissolution Reagent, 11 µl |
| 7 | 2-Mercaptethanol | Reagent for cell lysis, 0.2 µl |
| 8 | Glycogen | DNA Co-Precipitation Reagent, 0.2 µl |

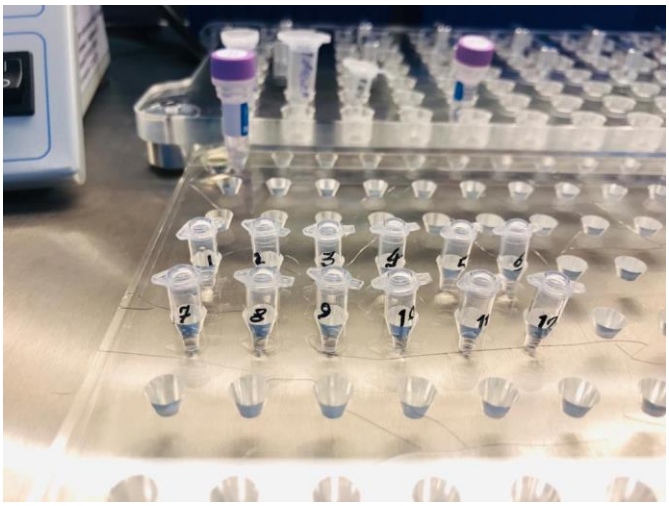


Figure 8. - Test tubes with nematodes for DNA extraction

The DNA extraction process consisted of the following steps (Figure 8):

1. First, the number of test tubes with a volume of 1.5 or 2 µl following the number of analyzed samples was written and put into an additional test tube for negative control. In all tubes (except negative), add 5-10 nematodes.

2.1. 20 µl of Lysing Solution 2 (No. 2) is added to each tube, and the nematodes are ground as much as possible in the microtube with a Teflon pestle. Then 280 l of No. 2 Lysing Solution and 1 l of No. 7 2-mercaptoethanol were added.

2.2. 10 µl of proteinase K (No.1) was added to the tubes and mixed in the vortex.

2.3 The samples are now left in the thermometer overnight at 56 ° C.

**Second day**

3.1. 100 µl of solution (No. 3) was added to the samples. The contents of the tubes were mixed in the vortex for 20 seconds.

3.2. The mixture was centrifuged at 13,000 circle for five minutes. At this point, the tube's bottom develops with a dense precipitate.

4.1. In clean 1.5-2 ml, tubes added 2 µl DNA co-precipitator (No.8).

4.2. The supernatant containing the DNA was transferred into test tubes.

4.3. 300 µl of Precipitation Solution 2 (No. 4) was added and mixed by inversion (10–12 times) until a visible DNA precipitate appeared.

4.4. The mixture was centrifuged at 13,000 rpm. 5 minutes. Then the supernatant was drained.

5.1. Added 400 µl Wash Solution (No. 5) and mixed several times by turning to wash the DNA.

5.2. After that, tubes were centrifuged for 2 min at 13,000 rpm. Then carefully remove the supernatant.

5.3. The tube lid was opened and placed in the thermometer for 10-15 minutes at 37 ° C, until the alcohol had completely evaporated.

5.4. Then 50 µl of Elution Solution (No.6) was added. stirred and warmed at 65 degrees Celsius for 5 minutes until the DNA was dissolved

5.5 Finally, the DNA solution was kept at -20 ° C for long-term storage.

### 2.3.2. Sequencing and phylogenetic analysis

For sequencing and further phylogenetic analysis, a PCR product with new primers was used, followed by separation in a 1% agarose gel.

Gene JET gel extraction kit from Thermo Scientific was used to purify the PCR result after amplified, and the Genetic Analyzer AB-3500 was used to sequence it using the dideoxy Sanger procedure (Applied Biosystems, USA). On the NCBI BLAST website, the sequencing results were then compared with the GenBank genetic sequence database [115, 116].

Sequence alignment editor BioEdit v.7.0.5.3 was used to test, edit, and align the sequence. The two-parameter model proposed by Kimor was used to determine pairwise genetic differences between the sequences [38].

The Mega 11 software's maximum likelihood method (ML method) was used to create diagrams of trees. The tree diagrams' accuracy was examined and validated using the Bootstrap Test by creating 1000 alternative trees. The findings are displayed as the percentage of similarity between the DNA sequences produced in this investigation and those found in GenBank.

### 2.3.3 Primer creation

In this investigation, eight *D. destructor* ITS Sequences were amplified and uploaded in the Genbank database [38, 117] as MN307126, MN307128, MN658597, MN122076, MN658599, and MN65388637 (Table 2). For the creating species-specific

primer, these and other original sequences obtained from the studied populations, as well as sequences that other researchers have uploaded into the Genbank database, were used. In addition, a number of *D. dipsaci* sequences (MG676655, MG676656, MG676657, *D. gigas*: KJ653270, KJ653267 (Table 3) were used for alignment due to their strong connection genetically to *D. destructor*.

The rDNA-ITS nucleotide sequences were aligned with BioEdit7.0.5.3 to look for conserved regions. The designed dsn.1 F/ R primers should allow amplification of the 397 bp fragment. and dsn.2 F/R size of 330 b.p. The primer design was validated by BLAST [116] to rule out non-specific responses to interactions with similarly related species. Additionally, primer quality was assessed using Oligo 6.0 software.

Table 2. - Sequences of the studied populations of *Ditylenchus destructor* (numbers 1 to 8 were uploaded in the Genbank database) [116]

| № | Sequence identification number | Region (country) |
|---|---|---|
| 1 | MN307126.1 | Iran |
| 2 | MN307128.1 | Iran |
| 3 | MN493767.1 | Iran |
| 4 | MN122076.1 | Russian Federation |
| 5 | MN658637.1 | Russian Federation |
| 6 | MN658638.1 | Russian Federation |
| 7 | MN658597 | Russian Federation |

| № | Sequence identification number | Region (country) |
|---|---|---|
| 8 | MN658599 | Russian Federation |
| 9 | MN.I.2 | Iran |
| 10 | MN.I.3 | Iran |
| 11 | MN.B.2 | Iran |
| 12 | MN.B.3 | Iran |
| 13 | MN.C.2 | Iran |
| 14 | MN.C.3 | Iran |
| 15 | DNil.I.1 | Iran |
| 16 | DNil.I.2 | Iran |
| 17 | DNil.I.3 | Iran |
| 18 | MN.1.R.2 | Russian Federation |
| 19 | MN.1.R.3 | Russian Federation |
| 20 | MN.2.R.2 | Russian Federation |
| 21 | MN.2.R.3 | Russian Federation |
| 22 | MN.3.R.2 | Russian Federation |
| 23 | MN.3.R.3 | Russian Federation |

Table 3. - GenBank sequences of closely related *Ditylenchus* species for the development of species-specific primers [116, 123]

| Sequence identification number | Kinds | Country |
|---|---|---|
| MG676655 | *D. dipsaci* | Japan |
| MG676656 | *D. dipsaci* | Japan |
| MG676657 | *D. dipsaci* | Japan |
| KJ653270 | *D. gigas* | Iran |
| KJ653267 | *D. gigas* | Iran |

### 2.3.4. Using species-specific primers for PCR

The composition of the reaction mixture is shown in Table 2. The final volume of the reaction mixture is 25 µl (Table 4, 5).


Figure 9. - PCR Veriti 96 well thermal cycler, Applied Biosystem

For species-specific PCR amplification, primer sets dsn.1 F/R and dsn.2 F/ R were used (Table 3). The contents were gently mixed by shaking. The reaction proceeded in a thermostat with the following step-by-step procedure: DNA was first annealed at 60.32°C (dsn.1 F/R) and 57.73°C (dsn.2 F/R) for 35 seconds, then at 95°C (dsn.1 F/R) for 3 minutes, annealed at 60.32°C (dsn.1 F/R) for 35 seconds, then annealed at 95°C (dsn.1 F/R) for 35 seconds [116].

Each amplification kit came with a bad control.

Following that, the PCR products were sorted by size using a 1 percent analyzed by agarose gel electrophoresis, documented by gel analysis, or purified for sequencing [116] (Figure 9).

Table 4. - Composition of the PCR reaction mixture (For two developed primers) [116]

| Reagents | Volume | Reagents | Volume |
|---|---|---|---|
| Master mix | 5,0 µl | Master mix | 5,0 µl |
| Primers dsn.1 F | 0,6 µl | Primers dsn.2 F | 0,6 µl |
| Primers dsn.1 R | 0,6 µl | Primers dsn.2 R | 0,6 µl |
| $H_2O$ | 13,8 µl | $H_2O$ | 13,8 µl |
| DNA | 5,0 µl | DNA | 5,0 µl |
| Overall volume | 25,0 µl | Overall volume | 25,0 µl |

Table 5. - Description of the first primer set

| Primer | Sequence (5′–3′) | Lenghth of DNA |
|---|---|---|
| dsn.1 F | TTGGCACGTCTGATTCAGGG | ITS rDNA |
| dsn.1 R | GTCAACATTGGCCAAGAGG C | ITS rDNA |
| dsn.2 F | TTTCGAATGCACATTGCGCC | ITS rDNA |
| dsn.2 R | CTAGGCCAAAGAGACAGCG G | ITS rDNA |

### 2.3.5. Gel electrophoresis

After PCR, the samples were analyzed by agarose gel electrophoresis according to the following procedure.

A melted gel was prepared by dissolving 500 mg of agarose in 0.5 µl of 1X TBE buffer followed by heating in a microwave oven. Ethidium bromide was added to the melted and cooled to 60°C gel to a concentration of 0.5 µg/mL. The solution was thoroughly mixed. While the agarose solution was cooling, a suitable comb was selected to form sample slits in the gel and placed inside the electrophoresis unit.

The warm agarose solution was poured into the mold in which the comb was placed. The gel was allowed to solidify completely (30-45 minutes at room temperature), then a small amount of electrophoresis buffer was sprinkled over the gel and the comb was carefully removed. The required amount of electrophoresis buffer was poured into the electrophoresis chamber to cover the gel to a depth of about 1 mm. 5 µl of the PCR product was combined with 2 µl of the required 6x gel loading buffer.

A disposable micropipette was used to load the sample mixture into the slots of the immersed gel. Next, a voltage of 150 V was applied to the anode for 30-60 minutes at room temperature, which ensured the movement of the amplicon fragments to the positive cathode. Depending on the predicted size of the amplicon, their speed of movement is different, and they are distributed in the gel according to their molecular weight. The gel was first visualized with a UV detector and then captured using the NTAS® Computerized Gel Image Storage System using GDS version 3.32 software and saved to disk as TIFF files (Figure 10).

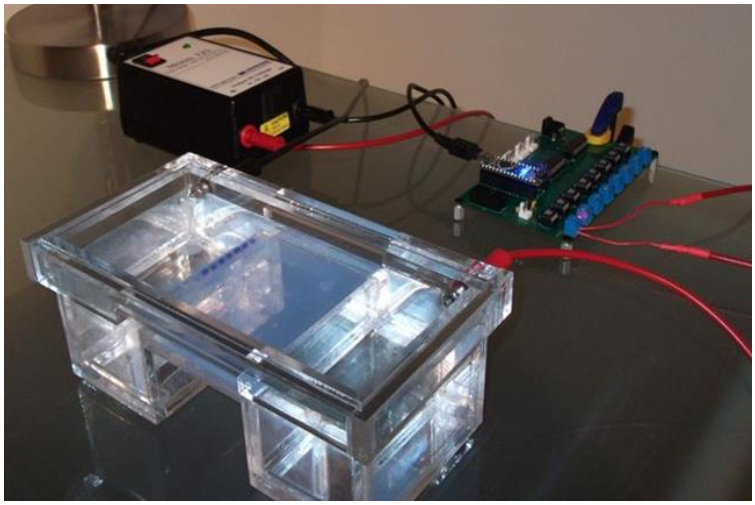


Figure 10. - Electrophoresis

### 2.3.6. Purification of PCR products

Thermo Scientific GeneJET was used to isolate and purify the PCR sample. After electrophoresis, the PCR products were visualized with a UV lamp, and a strip of gel was cut out using a sharp scalpel or razor blade, 2 mm wider than the strip on each side. The cut fragment was placed in the extraction buffer (Binding Buffer) in a ratio of 1:1. After the addition, mix thoroughly and transfer the solution to a GeneJET purification column. Centrifuged for 30-60 s. The resulting filtrate was removed. The GeneJET purification column was filled with 700 µl of wash buffer and centrifuged for 30-60 seconds. The resulting extract was removed and the purification column was placed back into the collection tube. After that, a clean 1.5 ml microcentrifuge tube was

used to transfer the cleaned GeneJET column, and centrifugation was continued for another minute. 50 µl of elution buffer were added to the membrane's middle of the GeneJET purification column and centrifuged for 1 min: the top solution was removed with a micropipette. For analysis, the lower solution was used, for which it was taken into new test tubes and numbered. Measurements of the DNA concentration in the sample were carried out by applying 2 µl of each sample to the device and processing it by a computer program. The Gene JET purification column was removed and the filtered PCR product was stored at -20°C.2.3.7.

### 2.3.7. Checking the concentration of nucleic acids

The NanoDrop spectrophotometer from NanoDrop Technologies is designed to measure the concentration of nucleic acids in 1 µl samples. The following procedure was used: one microliter of bidistilled water was loaded onto the lower optical surface. The lever arm was closed several times to wash the upper optical surface. Then the lever arm was raised and both optical surfaces were cleaned with a soft cloth or napkins. The Nano-Drop software was utilized to define the level of nucleic acid molecules. A zero measurement was performed by loading 1 µl of bidistilled water. After completion of the preparation, the optical surfaces were completely cleaned.

A 1 µl nucleic acid sample was placed on the detector and "measurement" was selected. At the end of the test, both optical surfaces were washed (Figure 11).


Figure 11. - Spectrophotometer Nanodrop 2000

# Chapter 3. RESULTS AND DISCUSSION

## 3.1. Results of morphological studies

A morphological comparison of *D. destructor* from the potato in the fields of the Russian Federation (Nizhny Novgorod, Bryansk, and Moscow regions) and samples collected from 13 settlements in Iran (Hamadan, Isfahan, Zanjan, and Ardebil provinces) was carried out (Figure 12, 13).

*D. dipsaci* and *D. destructor* were given a new procedure by [22]. This genus has between 67 and 80 species [10, 25]. Nevertheless, due to extremely minute changes between species, identification can occasionally be difficult. The adults of *D. destructor* are tiny, worm-like creatures that exhibit significant morphometric variation depending on the host and age.

In terms of appearance, males and females are comparable. Six incisures on the lateral field, two on the neck and tail. Cuticular and head annulation are fine; the head is often narrower than the neighboring body, and scanning microscopy can identify about four head annules [72]. Style cone with pronounced, rounded, and backward-sloping knobs that is 45 to 50 percent of the style length. Muscular medial bulb with about three-meter-long lumen wall thickenings.

On the dorsal bodyside, the posterior bulb briefly crosses the gut, albeit occasionally specimens have an offset glandular bulb. Excretory hole situated across from the esophageal glands.

The postvulval sac covers approximately three-quarters vulva-anus distance. Eggs that are twice as wide as they are [72, 80].

Vulva's lips are thick and raised. Outstretched anterior ovary that can occasionally extend to the oesophageal area. *D. dipsaci* has a ventral tumulus in the area of the calomus, which is different from *D. destructor* in terms of spiculum morphology [10, 80].

Testicles extended as they near the oesophageal base. Both sexes have a conical tail that is three to five anal body widths long, frequently curved in the ventricle, and

has a rounded termination. Although *D. destructor* and *D. dipsaci* are related, the latter is distinguished from the former by the lateral field's six incisions, longer postvulval sac, and finely rounded tail end.

However, none of these are pathogenic to potatoes, unlike *D. arachis* [14, 40], *D. afronus* [73]. *D. halictus* [73]. *D. longicauda* [76] and *D. oncogenus* [13, 77].

Additionally, the *D. destructor* can be distinguished from the *D. arachis* and *D. africanus* by its relatively longer spicules and distinct host preferences, by the *D. convallariae's* distinctive tail tip form, by the *D. halictus'* relatively longer body, relatively larger spicules, and by the *D. longicauda's* relatively larger spicules, higher PUS / VBW ratio, and distinct tail shape.

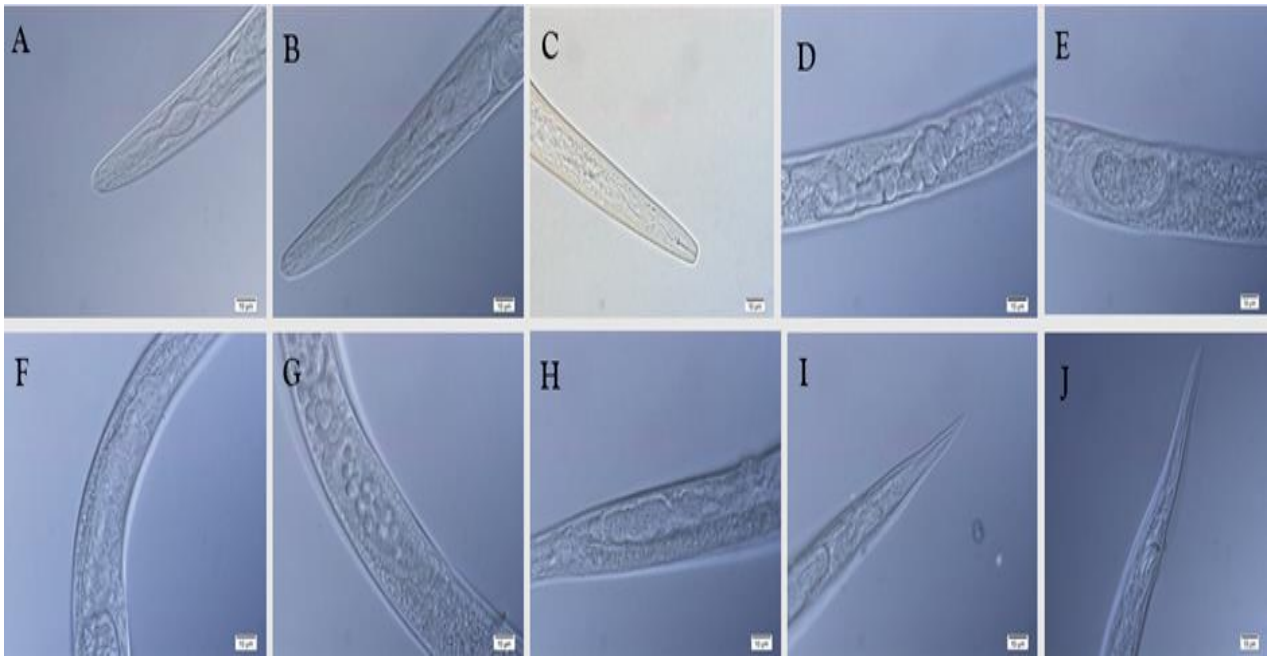



Figure 12. - Morphology of characteristic of *D. destructor* on the example of the Moscow population. A. Female anterior region. B. Oesophagus. C. stylet. D. crustaformeria (preuteral gland). E. Egg. F. Pus. G. Spermatheca. H. Vulva to anus distanc. I. Femail tail. J. cloacal region

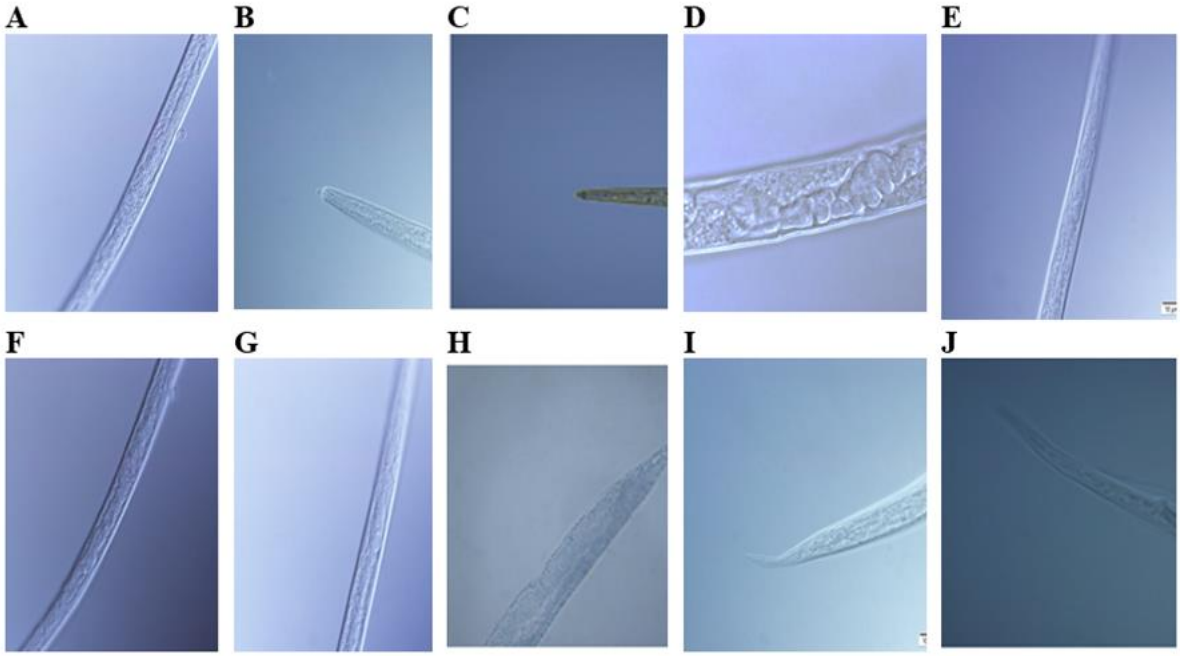



Figure 13.- Morphology of characteristic of *D. destructor* on the example of Iranian populations. A. Female anterior region. B. Oesophagus. C. stylet. D. crustaformeria (preuteral gland). E. Egg. F. Pus. G. Spermatheca. H. Vulva to anus distanc. I. Femail tail. J. cloacal region

It can be noted that the morphometric characteristics of the nematodes reported here (Table 6 to 14) for the regions of Hamedan, Isfahan, Zanjan, and Ardebil are comparable with the data by looking at the species' morphological and morphometric traits [11] (Table 15).

There are some variations from those described for *D. destructor* in the Nizhny Novgorod, Bryansk, and Moscow regions [20, 51, 94] (Table 16).

Table 6. - Russian Federation *Ditylenchus destructor* population’s morphology, µm

and min-max values (100 individuals)

| | (♂) n=50 | (♂) n=50 | (♂) n=50 | (♀) n=50 | (♀) n=50 | (♀) n=50 |
|---|---|---|---|---|---|---|
| Parameter | Briansk | Moscow | Nizhny Novgorod | Briansk | Moscow | Nizhny Novgorod |
| Body L | (890-1340) | (835-1550) | (918-1350) | (900-1664) | (954-1600) | (916-1571) |
| Tail L | (64-80) | (63-79) | (65-81) | (63-80) | (63-81) | (62-81) |
| c (body L/tail L) | (14-17) | (14-20) | (14-16) | (15-20) | (16-20) | (15-20) |
| Stylet L | (10-13) | (10-14) | (10-14) | (10-14) | (10-14) | (10-14) |
| Pus | – | - | – | (95-120) | (95-125) | (95-112) |
| Pus/Anus-Vulva distance% | – | - | – | (54-90) | (53-88) | (55-86) |
| Spicule L | (20-24) | (20-25) | (20-25) | – | - | _ |
| Bursa L | (37-58) | (34-62) | (39-79) | _ | - | _ |
| Tail shape | Rounded | Rounded | Rounded | Rounded | Rounded | Rounded |

Table7. - Population morphometry of *Ditylenchus destructor* samples from Iran, Hamedan Province (P1. Hamedan, P.2 Bahar, P3. Asad Abad, P4. Kabudarahang), µm and min-max values (P1: 50, P2: 50, P3: 50, P4: 50 individuals Males)

| Parameter (♂) Hamedan n: 200 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| Body L | (650 -1100) | (600-1200) | (650-1000) | (730-1100) |
| Tail L | (50-63) | (50-80) | (54-65) | (53-70) |
| c (body L/tail L) | (13-18) | (12-15) | (12-16) | (14-16) |
| Stylet L | (9-12) | (9-12) | (9-12) | (8 -11) |
| Pus L | – | – | - | - |
| Pus/Anus-Vulva distance % | – | – | - | - |
| Spicule L | (18-20) | (18-21) | (19-26) | (19-21) |
| Bursa L | (33-55) | (33-45) | (35-51) | (32-48) |
| Tail shape | Rounded | Rounded | Rounded | Rounded |

Table 8. - Population morphometry of *Ditylenchus destructor* samples from Iran, Isfahan Province (P1. Isfahan, P2. Mobarake, P3. Najafabad, P4. Felaverzhan), µm and min-max values (P1: 50, P2: 50, P3: 50, P4: 50 individuals Males)

| Parameter (♂) Isfahan n:200 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| Body L | (600-1250) | (637-1205) | (840-1200) | (600-1000) |
| Tail L | (45-76) | (52-71) | (67-80) | (51-67) |
| c (body L/tail L) | (14-17) | (13-17) | (13-15) | (12-15) |
| Stylet L | (9-12) | (9-12) | (9-11) | (9-11) |
| Pus | – | – | - | - |
| Pus/Anus-Vulva distance % | – | – | - | - |
| Spicule L | (18-21) | (19-27) | (19-21) | (18-21) |
| Bursa L | (33-52) | (33-56) | (31-56) | (33-52) |
| Tail shape | Rounded | Rounded | Rounded | Rounded |

Table 9. - Population morphometry of *Ditylenchus destructor*, samples from Iran, Zanjan Province (P1. Zanjan, P2. Soltaniye), µm and min-max values (P1: 100, P2: 100, males)

| Parameter (♂) Zanjan n: 200 | P1 | P2 |
|---|---|---|
| Body L | (700-1200) | (850-1167) |
| Tail L | (50-66) | (61-75) |
| c (body L/tail L) | (14-19) | (14-16) |
| Stylet L | (9-12) | (9-11) |
| Pus | – | - |
| Pus/Anus-Vulva distance % | – | - |
| Spicule L | (18-23) | (19-27) |
| Bursa L | (33-56) | (35-58) |
| Tail shape | Rounded | Rounded |

Table 10. - Population morphometry of *Ditylenchus destructor* samples from Iran, Ardebil Province (P1. Ardebil, P2. Namin, P3. Nayer), µm and min-max values (P1: 100, P2: 50, P3:50 individuals Males)

| Parameter (♂) Ardebil n: 200 | P1 | P2 | P3 |
|---|---|---|---|
| Body L | (750-1100) | (600-1205) | (640-1164) |
| Tail L | (57-75) | (44-73) | (47-72) |
| c (body L/tail L) | (13-15) | (14-17) | (14-17) |
| Stylet L | (9-12) | (8-13) | (8-13) |
| Pus | – | – | - |
| Pus/Anus-Vulva distance % | – | – | - |
| Spicule L | (18-27) | (19-24) | (18-26) |
| Bursa L | (33-50) | (35-55) | (33-56) |
| Tail shape | Rounded | Rounded | Rounded |

Table 11. - Population morphometry of *Ditylenchus destructor*, samples from Iran, Hamedan Province (P1. Hamedan, P2. Bahar, P3. Asad Abad, P4. Kabudarahang), µm and min-max values (P1: 50, P2: 50, P3: 50, P4: 50 females)

| Parameter (♀) Hamedan n: 200 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| Body L | (875 -1110) | (809-1200) | (900-1400) | (980-1256) |
| Tail L | (54-71) | (78-83) | (55-64) | (81-85) |
| c (body L/tail L) | (14-16) | (11-15) | (17-22) | (12-15) |
| Stylet L | (8-13) | (9-13) | (8-12) | (8-12) |
| Pus | (53-98) | (53-89) | (53-90) | (53-87) |
| Pus/Anus-Vulva distance % | (48-71) | (42-87) | (55-91) | (44-75) |
| Spicule L | - | - | - | - |
| Bursa L | - | - | - | - |
| Tail shape | Rounded | Rounded | Rounded | Rounded |

Table 12. - Population morphometry of *Ditylenchus destructor* samples from Iran, Isfahan Province (P1. Isfahan, P2. Mobarake, P3. Najafabad, P4. Felaverzhan), µm and min-max values (P1: 50, P2: 50, P3: 50, P4:50 individuals Females)

| Parameter (♀) Isfahan n: 200 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|
| Body L | (800-1100) | (770-1300) | (700-1200) | (820-1310) |
| Tail L | (55-63) | (55-79) | (58-76) | (54-75) |
| c (body L/tail L) | (15-18) | (14-17) | (12-16) | (15-18) |
| Stylet L | (9-12) | (9-13) | (9-13) | (9-12) |
| Pus | (55-98) | (55-103) | (54-105) | (52-98) |
| Pus/Anus-Vulva distance % | (38-86) | (37-79) | (37-90) | (37-87) |
| Spicule L | - | - | - | - |
| Bursa L | - | - | - | - |
| Tail shape | Rounded | Rounded | Rounded | Rounded |

Table 13. - Population morphometry of *Ditylenchus destructor*, samples from Iran, Zanjan Province (P1. Zanjan, P2. Soltaniye), µm and min-max values (P1: 100, P2: 100 individuals Females)

| Parameter (♀) Zanjan n: 200 | P1 | P2 |
|---|---|---|
| Body L | (880-1200) | (700-1114) |
| Tail L | (65-76) | (58-80) |
| c (body L/tail L) | (14-16) | (12-14) |
| Stylet L | (9-13) | (8-13) |
| Pus | (55-86) | (58-88) |
| Pus/Anus-Vulva distance % | (45-86) | (37-87) |
| Spicule L | - | - |
| Bursa L | - | - |
| Tail shape | Rounded | Rounded |

Table 14. - Population morphometry of *Ditylenchus destructor*, samples from Iran, Ardebil Province (P1. Ardebil, P2. Namin, P3. Nayer), µm and min-max values (P1: 100, P2: 50, P3: 50 females)

| Parameter (♀) Ardebil n: 200 | P1 | P2 | P3 |
|---|---|---|---|
| Body L | (800-1200) | (760-1200) | (640-1300) |
| Tail L | (52-69) | (46-65) | (43-78) |
| c (body L/tail L) | (16-18) | (17-19) | (15-17) |
| Stylet L | (8-12) | (8-13) | (8-12) |
| Pus | (55-88) | (54-89) | (53-100) |
| Pus/Anus-Vulva distance % | (45-83) | (52-78) | (42-76) |
| Spicule L | - | - | - |
| Bursa L | - | - | - |
| Tail shape | Rounded | Rounded | Rounded |

Table 15. - Morphometry of *Ditylenchus destructor* from Iran according to [11]

| Parameter | (♂) | (♀) |
|---|---|---|
| Body L | (600–1254) | (603–1468) |
| Tail L | (45–87.5) | (39.5–90.5) |
| c (body L/tail L) | (11.0–19.1) | (11.4–27.8) |
| Stylet L | (8.5–12) | (8–13) |
| Pus/Anus-Vulva distance % | - | (36.9– 92.6) |
| Spicule L | (18–28) | - |
| Tail shape | Rounded/Pointed | Rounded/Pointed |

Table 16. - Morphometry of *Ditylenchus destructor* according to [20,51,94]

| | | (♂) | | | (♀) | |
|---|---|---|---|---|---|---|
| Parameter | Hooper (1973) | Thorne (1945) | Chizhov (2010) | Hooper (1973) | Thorne (1945) | Chizhov (2010) |
| Body L | (800-1900) | (750-1300) | (860-1370) | (800-1900) | (720-1440) | (1050-1630) |
| Tail L | - | - | (62-81) | - | - | (62-81) |
| c (body L/tail L) | (14-20) | (12-16) | (13.6-16.2) | (14-20) | (15-20) | (13.8-18.8) |
| Stylet L | (10-14) | (10) | (10-13) | (10-14) | (10) | (11-13) |
| Pus/Anus-Vulva distance % | - | - | - | (53-90) | - | - |
| Spicule L | (24-27) | (24) | (20-25) | - | - | - |
| Tail shapre | Rounded | Rounded | - | Rounded | Rounded | - |

**Males**

The body length of *D. destructor* males from each population was (μm): 1. Nizhny Novgorod region (918-1350); 2. Bryansk region (890-1340); 3. Moscow region (835-1550); 4. Hamedan Province: P1. (650-1100), P2. (600-1200), P3. (650-1000), P4. (730-1100); 5. Isfahan Province: P1. (600-1250), P2. (637-1205), P3. (840-1200), P4. (600-1000); 6. Zanjan Province: P1. (700-1200), P2. (850-1167) ;7. Ardebil Province: P1. (800-1200), P2. (760-1300), P3. (640-1164), respectively. The shortest body length was found in the population from Hamadan Province, Isfahan and Ardabil, and the longest in the populations of the Moscow region.

The body length of the populations from Hamedan Province, Isfahan and Zanjan corresponded to the body length of those measured [11] (Table 15). In terms of body length, populations from the Bryansk region correspond [94], Moscow region [51], and Nizhny Novgorod region [94, 51], respectively (Table 16).

Comparison of tail length in *D. destructor* males in each population was (μm): 1. Nizhny Novgorod region (65-81); 2. Bryansk region (64-80); 3. Moscow region (63-79); 4. Hamedan Province: P1. (50-63), P2. (50-80), P3. (54-65), P4. (53-70); 5. Isfahan Province: P1. (45-76), P2. (52-71), P3. (67-80), P4. (51-67); 6. Zanjan Province: P1. (50-66), P2. (61-75).

Ardebil Province: P1. (52-69), P2. (46-65), P3. (43-78), respectively. The shortest tail length was observed in the population from the province of Ardebil, and the longest in populations from the Nizhny Novgorod region. The tail length of all Russian populations corresponds to [94] (Table 16).

Parameter "C" of the ratio of body length to tail length in *D. destructor* males from each population was: 1. Nizhny Novgorod region (14-16); 2. Bryansk region (14-17); 3. Moscow region (14-20); 4. Hamadan Province: P1. (13-18), P2. (12-15), P3. (12-16), P4. (14-16); 5. Isfahan Province: P1. (14-17), P2. (13-17), P3. (13-15), P4. (12-15); 6. Zanjan Province: P1. (14-19), P2. (14-16) ;7. Ardebil Province: P1. (16-18), P2. (17-19), P3. (15-17).

The shortest "C" score was calculated for populations from the Hamadan Province, and the longest for populations from the Moscow region. The “C” index for the population from Hamadan, Isfahan, Zanjan and Ardebil provinces were at the same level and corresponded to [11].

According to C, the populations from the Bryansk region correspond to those of the Moscow region [51] and the Nizhny Novgorod region [94, 51], respectively (Table 16).

The stylet length in *D. destructor* males in each population was (μm): 1. Nizhny Novgorod region (10-14); 2. Bryansk region (10-13); 3. Moscow region = (10-14); 4. Hamedan Province: P1. (9-12), P2. (9-12). P3. (9-12), P4. (8-11); 5. Isfahan Province: P1. (9-12), P2. (9-12), P3. (9-11), P4. (9-11), 6. Zanjan Province: P1. (9-12), P2. (9-11) ;7. Ardebil Province: P1. (9-12), P2. (8-13), P3. (8-13), respectively.

The shortest stylet length is for the regions of Hamedan and Ardebil, the longest is for Nizhny Novgorod and Moscow. In populations from Hamedan Province, Isfahan, and Zanjan, the stylet length was consistent with [11] (Table 15). The stylet length of the populations from the Bryansk region corresponds [94], the Moscow region and the Nizhny Novgorod region [51] (Table 16).

Comparison of the spicule length in *D. destructor* males showed that in populations from 1. Nizhny Novgorod (20-25), 2. Bryansk (20-24), 3. Moscow regions (20-25), 4. Hamedan province: P1. (18-20), P2. (18-21), P3. (19-26), P4. (19-21), 5. Isfahan Province: P1. (18-21), P2. (19-27), P3. (19-21), P4. (18-21), 6. Zanjan Province: P1. (18-23), P2. (19-27), 7. Ardebil Province: P1. (18-27), P2. (19-24), P3. (18-26). The shortest spicules and Iran has the longest.

Additionally, in the population from the regions of Hamadan, Isfahan, Ardebil and Zanjan, the length of the spicules corresponded to [11] (Table 15), and for the populations from the Nizhny Novgorod, Bryansk and Moscow regions they corresponded to [94] (Table 16).

The length of the bursa in *D. destructor* males in each population was (μm): 1. Nizhny Novgorod region (39-79); 2. Bryansk region (37-58); 3. Moscow region (34-62); 4. Hamedan Province: P1. (33-55), P2. (33-45), P3. (35-51), P4. (32-48); 5. Isfahan Province: P1. (33-52), P2. (33-56), P3. (31-56), P4. (33-52); 6. Zanjan Province: P1. (33-56), P2. (35-58).

Ardebil Province: P1. (33-50), P2. (35-55), P3. (33-56), respectively. The shortest bursa was noted in populations from the Isfahan Province, and the longest in populations from the Nizhny Novgorod and Moscow regions.

**Females**

The body length of *D. destructor* females for populations from different regions was (in μm): 1. Nizhny Novgorod region (916-1571); 2. Bryansk region (900-1664); 3. Moscow region (954-1600); 4. Hamedan Province: P1. (875-1110), P2. (809-1200), P3.

(900-1400), P4. (980-1256); 5. Isfahan Province: P1. (800-1100), P2. (770-1300), P3. (700-1200), P4. (820-1310); 6. Zanjan Province: P1. (880-1200), P2. (700-1114) ;7. Ardebil Province: P1. (800-1200), P2. (760-1200), P3. (640-1300). The shortest body length was noted in individuals from Ardeil, the longest from the Bryansk region.

The body length of the populations in Hamedan Province, Isfahan and Zanjan was consistent with the data [11] (Table 15). The body length of the population from Nizhny Novgorod, Bryansk and the Moscow region corresponded to [51] (Table 16).

The tail length of *D. destructor* from each population, females were (in μm): 1. Nizhny Novgorod region (62-81); 2. Bryansk region (63-80); 3. Moscow region (63-81); 4. Hamedan Province: P1. (54-71), P2. (78-83), P3. (55-64), P4. (81-85); 5. Isfahan Province: P1. (55-63), P2. (55-79), P3. (58-76), P4. (54-75); 6. Zanjan Province: P1. (65-76), P2. (58-80).

Ardebil Province: P1. (52-69), P2. (46-65), P3. (43-78). The shortest tail length was recorded in individuals from Ardeil Province, and the longest from Hamadan Province. The length of the populations in the provinces of Hamedan, Isfahan and Zanjan corresponded to the data [11] [Table 15]. Populations from the Nizhny Novgorod, Bryansk and Moscow regions corresponded to [94] (Table 16).

Comparison of C (ratio of body length to tail length) in *D. destructor* females of each population was carried out (in μm): 1. Nizhny Novgorod region (15-20); 2. Bryansk region (15-20); 3. Moscow region (16-20); 4. Hamedan Province: P1. (14-16), P2. (11-15), P3. (17-22), P4. (12-15); 5. Isfahan Province: P1. (15-18), P2. (14-17), P3. (12-16), P4. (15-18); 6. Zanjan Province: P1. (14-16), P2. (12-14);7. Ardebil Province: P1. (16-18), P2. (17-19), P3. (15-17).

The shortest C belongs to Isfahan and Hamedan and the longest belongs to Hamedan Province. Indicator C throughout the Hamadan Provinces, Isfahan, Zanjan and Ardebil was in line with [11] (Table 15). The populations of the Nizhny Novgorod, Bryansk and Moscow regions corresponded to [20, 51] (Table 16).

The length of the stylet in *D. destructor* females of each population was (in μm): 1. Nizhny Novgorod region (10-14); 2. Bryansk region (10-14); 3. Moscow region (10-14); 4. Hamedan Province: P1. (8-13), P2. (9-13), P3. (8-12), P4. (8-12); 5. Isfahan Province: P1. (9-12), P2. (9-13), P3. (9-13), P4. (9-12); 6. Zanjan Province: P1. (9-13), P2. (8-13).

Ardebil Province: P1. (8-12), P2. (8-13), P3. (8-12). The longest stylet length throughout the Russian Federation's regions. The stylet length of the populations from Hamedan, Isfahan, Zanjan and Ardebil provinces corresponded to the data [11] (Table 15). The populations of the Nizhny Novgorod, Bryansk and Moscow regions corresponded to [94, 51] (Table 16).

Comparison Pus [120] *D. destructor* of each population was carried out (in microns): 1. Nizhny Novgorod region (95-112); 2. Bryansk region (95-120); 3. Moscow region (95-125); 4. Hamedan Province: P1. (53-98), P2. (53-89), P3. (53-90), P4. (53-87); 5. Isfahan Province: P1. (55-98), P2. (55-96), P3. (54-98), P4. (52-98); 6. Zanjan Province: P1. (55-86), P2. (58-88);7. Ardebil Province: P1. (55-88), P2. (54-89), P3. (53-98). The shortest Pus belonged to the Isfahan Province, and the longest to the Moscow Region.

Pus / Anus-Vulva distance % in *D. destructor* females in each each population was carried out (in microns): 1. Nizhny Novgorod region (55-86); 2. Bryansk region (54-90); 3. Moscow region (53-88); 4. Hamedan Province: P1. (48-71), P2. (42-87), P3. (55-91), P4. (44-75); 5. Isfahan Province: P1. (38-86), P2. (37-79), P3. (37-90), P4. (37-87); 6. Zanjan Province: P1. (45-86), P2. (37-87);7. Ardebil Province: P1. (45-83), P2. (52-78), P3. (42-76).

The shortest Pus / Anus-Vulva distance % belonged to Isfahan and Zanjan Province, while the longest belonged to Hamedan Province. The populations of the Nizhny Novgorod, Bryansk and Moscow regions corresponded to [51] (Table 16).

The morphology of the populations was examined, and it was discovered that every population under investigation belonged to the species *D. destructor*. The size of individual characteristics in the populations from Iran and Russia differ slightly, as was reported, however this variability has no effect on whether or not all populations belong to the species *D. destructor*.

## 3.2. Results of molecular studies

### 3.2.1. DNA sequence analysis of *Ditylenchus destructor*

350 units of Nematodes were gathered for molecular investigation from seven locations in the Russian Federation and Iran. After alignment (Mega 11 software) and cleaning and editing sequences with BioEdit 7.0.5.3 software, we selected 56 sequences (meaning 8 sequences from each area), but we submitted only eight sequences from all areas to Genbank, as well as for the volume of sequences, a total of three for each region indicated in the result of the dissertation and phylogenetic tree. All of the *D. destructor* DNA sequences from Russia and Iran retrieved for this investigation matched the relevant *D. destructor* sequences found in the database, according to a BLAST search at NCBI. Sequence comparisons between *D. destructor* isolates from several host plants showed sequence variation across distinct geographic populations.

### 3.2.2. Phylogenetic studies of *Ditylenchus destructor* populations

Phylogenetic analyses within *D. destructor* populations were performed based on the *D. destructor* sequences obtained in this study and closely related species available from the GenBank (Tables 17 and 18).

Table 17. - *Ditylenchus destructor* sequences used for phylogenetic analysis [116]

| Registration number | Region | Plants |
|---|---|---|
| EU400627 | South Korea | *Ipomoea batatas* |
| MH992393 | China | *Solanum tuberosum* |
| EF208213 | China | *Solanum tuberosum* |
| MG673926 | China | *Daucus carota subsp* |
| HQ235698 | Iran | *Solanum tuberosum* |
| EU400636 | China | *Ipomoea batatas* |
| FJ707365 | Czech Republic | *Solanum tuberosum* |
| EU400638 | China | *Ipomoea batatas* |
| MG675235 | China | *Daucus carota subsp* |
| EU400643 | China | *Ipomoea batatas* |
| KY435979 | China | *Daucus carota subsp* |
| EU400639 | China | *Ipomoea batatas* |
| GQ469490 | USA | *Solanum tuberosum* |

| Registration number | Region | Plants |
|---|---|---|
| JX162205 | Canada | *Allium sativum* |
| DQ328727 | Россия | *Solanum tuberosum* |
| JN166693 | Iran | *Solanum tuberosum* |
| MK979365 | China | *Solanum tuberosum* |
| LC030371 | Japan | *Solanum tuberosum* |
| GQ469491 | Czech Republic | *Solanum tuberosum* |
| DQ471335 | China | *Solanum tuberosum* |

Table 18. - *Ditylenchus dipsaci* sequences used for phylogenetic analysis [116]

| Registration number | Country | Host plant |
|---|---|---|
| GQ469497 | Czech Republic | *Solanum tuberosum* |
| MG676656 | Japan | *Solanum tuberosum* |
| KY348765 | Mexici | *Medicago sativa* |
| KT806479 | China | *Solanum tuberosum* |
| MG676655 | Japan | *Phlox subulata* |
| MG676657 | Japan | *Phlox subulata* |

Phylogenetic relationships within and between *Ditylenchus* species were estimated utilizing the concept of maximum likelihood. Despite sequence variability, the phylogenetic tree strongly supports the grouping of all *D. destructor* sequences, even for populations of different geographic origins and different host plants.

On the phylogenetic tree, *D. dipsaci* is used as a sister species to *D. destructor* and *Xiphinema* for species identification. The results of ITS-rRNA analysis revealed three main clades. Section I is divided into three subclasses.

The first one consists of sequences from *D. dipsaci* populations, the second one of *D. destructor* sequences from Russian populations, and the third shows *D. destructor* populations from other countries.

The second section consists of *D. destructor* populations collected from regions of Iran that have the most similarity to populations in China. Section 3 shows the population outside the group.

The results of our genetic analysis distinguish *D. destructor* from *D. dipsaci* and other recognized *Ditylenchus* species and are therefore consistent with previous studies [4, 94, 98, 101] and [120].

Some congruence in the *Ditylenchus* spp. phylogeny has also been observed, as previously demonstrated [101].

with their host species. As previously proposed, the ITS phylogeny suggests that *Ditylenchus* may be a paraphyletic taxon that contains a number of separate evolutionary lineages [98] (Figure 14).

Previous investigators have classified *D. dipsaci* and *D. destructor* into two taxonomic groups based on 18S rDNA, (2017), which is consistent based on the findings of the current investigation.

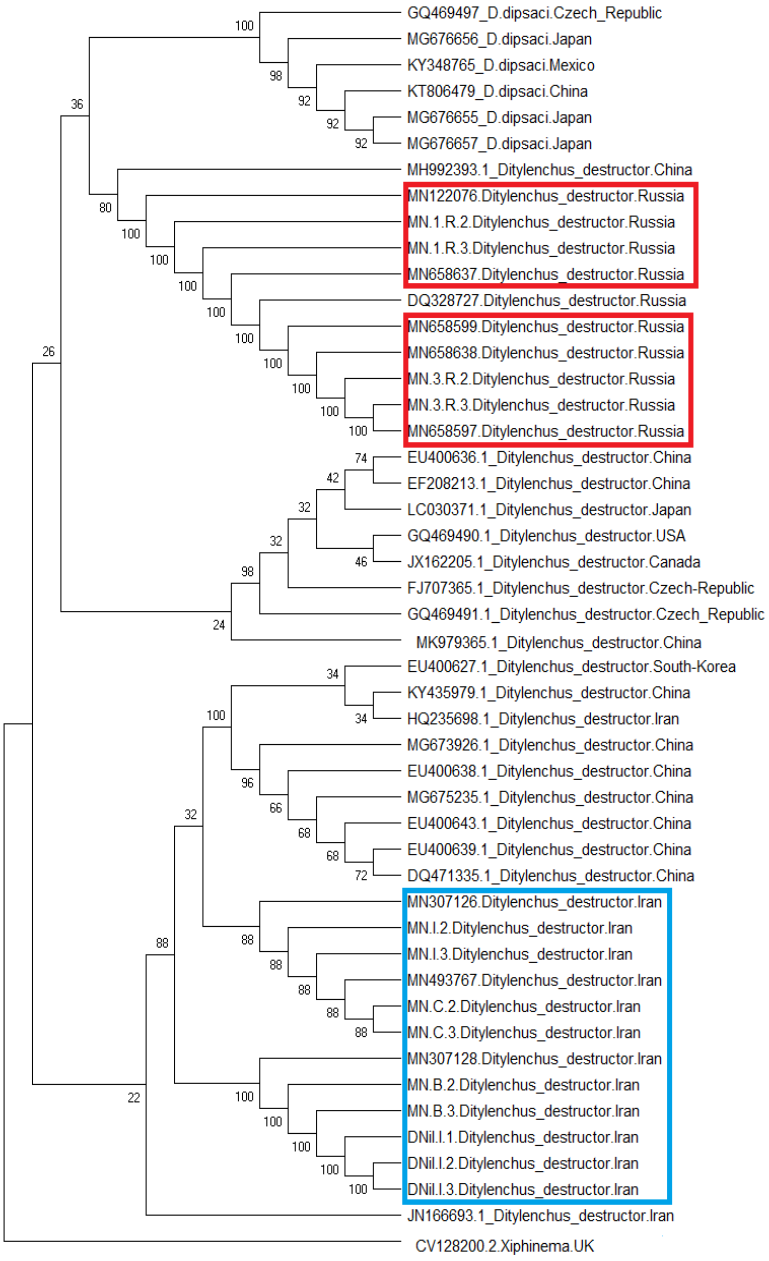



Figure 14. - Phylogenetic tree with studied populations and reference sequences (Red colors from Russia, Blue colors from Iran)

### 3.2.3. Development of the primers species-specific

*D. destructor*, *D. dipsaci*, and *D. gigas* were compared to the first primer, dsn.1, which was created to have some of the nucleotide mismatches found in those three species. NCBI-Primer Design (Table 20) (Figure 15, 16) developed the second primer, named dsn.2.

The reliability of primer specificity was tested on genomic DNA isolated from the studied populations. The standard conditions described for the PCR of the ITS regions were not changed for this particular amplification, but with a different annealing temperature.

PCR amplification with species-specific primers yields a specific and distinct PCR product band for one adult for each of the studied geographic populations of *D. destructor* that were gathered from the potato fields for our investigation.

*D. dipsaci*, *D. gigas*, and *D. destructor* have all been used in vitro to verify the primers' specificity and dependability. Diagnostic primers can offer a quick and accurate way to detect *D. destructor* individuals isolated from various host plants in various geographical locations due to the specificity, sensitivity, and dependability of *D. destructor* primers.


Figure 15. - For *Ditylenchus destructor* and other *Ditylenchus* species, a species-specific forward primer was constructed using repeated alignment of the rDNA-ITS sequences from GenBank. (forward primer starts at 193-212, reverse primer starts at 318-299)

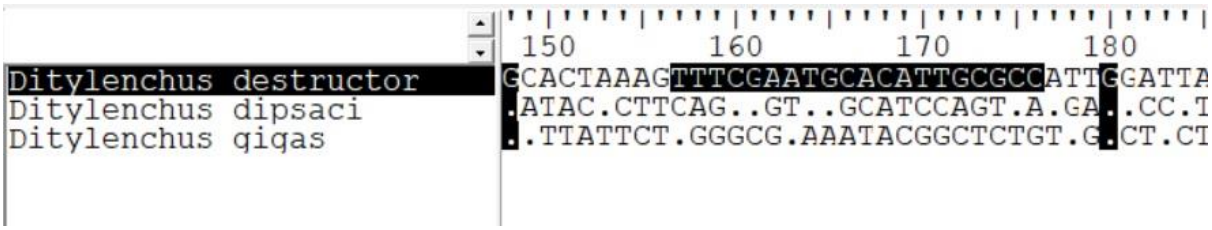



Figure 16. - For *Ditylenchus destructor* and other *Ditylenchus* species, a species-specific reverse primer was constructed using repeated alignment of the rDNA-ITS sequences from GenBank. (forward primer starts at 157-176, reverse primer starts at 281-262)

Based on the theoretically identified promising sequences, primers were designed and synthesized (Evrogen):

Table 19. - Description of the dsn.1 F/R species-specific primers for diagnosing *Ditylenchus destructor*

| Primer.dsn.1 F/R | Subsequence | Annealing temperature | Product size (bp) |
|---|---|---|---|
| Forward | TTGGCACGTCTGATTCAGGG | 60.3 | 397 |
| Reverse | GTCAACATTGGCCAAGAGGC | | |

Table 20. - Description of the dsn.2 F/R species-specific primers for diagnosing *Ditylenchus destructor*

| Primer.dsn.2 F/R | Subsequence | Annealing temperature | Product size (bp) |
|---|---|---|---|
| Forward | TTTCGAATGCACATTGCGCC | 57.7 | 330 |
| Reverse | CTAGGCCAAAGAGACAGCGG | | |

### 3.2.4. Using PCR primers with species-specificity

Seven nematode populations' DNA was subjected to PCR using species-specific primers. Successful amplification was achieved for all *D. destructor* accessions received from Iran and Russia.

All samples showed the same band at approximately 397 bp. for dsn.1 F/R and 330 b.p. for dsn.2 F/R. In all cases, the absence of non-specific products in the negative control samples was demonstrated.

The findings of this research demonstrate additional identification of *D. destructor* from various populations of this genus utilizing species-specific primers in PCR. The interpretation of the results can be improved by using high resolution agarose gels. Given the diverse host variety of the *D. destructor*, the use of using PCR primers with species-specificity greatly accelerates the screening of various types of agricultural products for the presence of this type of parasitic nematode. The potato stem nematode is a serious problem in modern agriculture, despite all phytosanitary methods for controlling its harmfulness during the seed potato production. The diagnosis of this species using PCR can speed up its detection in the early stages of production.

This, in turn, will make it possible to more quickly use various approaches to reduce the infection of potato seeds with nematodes and thereby reduce its harmful effects.

The present study successfully developed a method for qualifying the evaluation of *D. destructor* in potato tubers. The ITS sequences determined in this study were identical among nematodes collected from 7 different regions of the two countries, although the genetic diversity of *D. destructor* in sequence repeats between samples is relatively high. The NCBI database lists various sequences from various countries. These results suggest that the primer set developed in this study specifically amplifies the sequence present in *D. destructor* in all different regions.

There is another diagnostic tool for identifying economically important parasitic nematode species, such as *D. dipsaci*, *D. weischeri*, and *D. gigas*, and this is the PCR-RFLP method (PCR-RFLP) [4, 94, 97, 101].

This method cannot be used if the specimen has more than one species of nematode, but it is perfect for identifying species in specimens that are monospecific. The method developed in the current study for PCR primers with species-specificity overcomes this limitation and allows differentiation of target species. Compared to ITS-PCR-RFLP, the PCR approach reduces diagnostic time and costs.

### 3.2.5. New primer tests

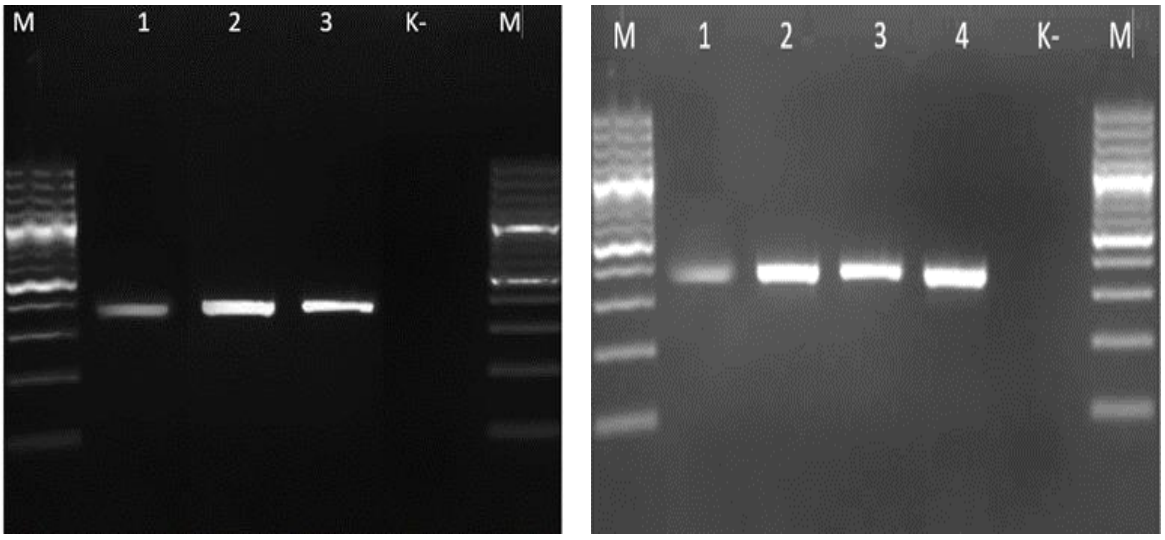
#### 3.2.5.1. Primer selectivity test

PCR amplification products for distinct *D. destructor* populations generated with use of two primer pairs (ds.1F/R and dsn.2F/R) were analyzed in terms of increasing the selectivity of the species-specific PCR primers.

The 397 and 330 bp fragments that made up the clear band were recovered (Figure 17, 18). The technique worked well for the precise identification of DNA samples from *D. destructor*. By obtaining the anticipated fragment sizes for all *D. destructor* populations and finding no products for the tested populations of non-target *D. dipsaci* and *D. gigas* species, particular primers' reliability and specificity were verified.

The primary purpose of this study was to design a set of species-specific primer pairs (dsn.1 F/R and dsn.2 F/R) for the molecular identification of *D. destructor* based on rDNA-ITS sequence analysis. In order to effectively manage nematodes, the created specialized primers should be quick and accurate molecular methods for identifying *D. destructor*.

**A** **B**



**C** **D**

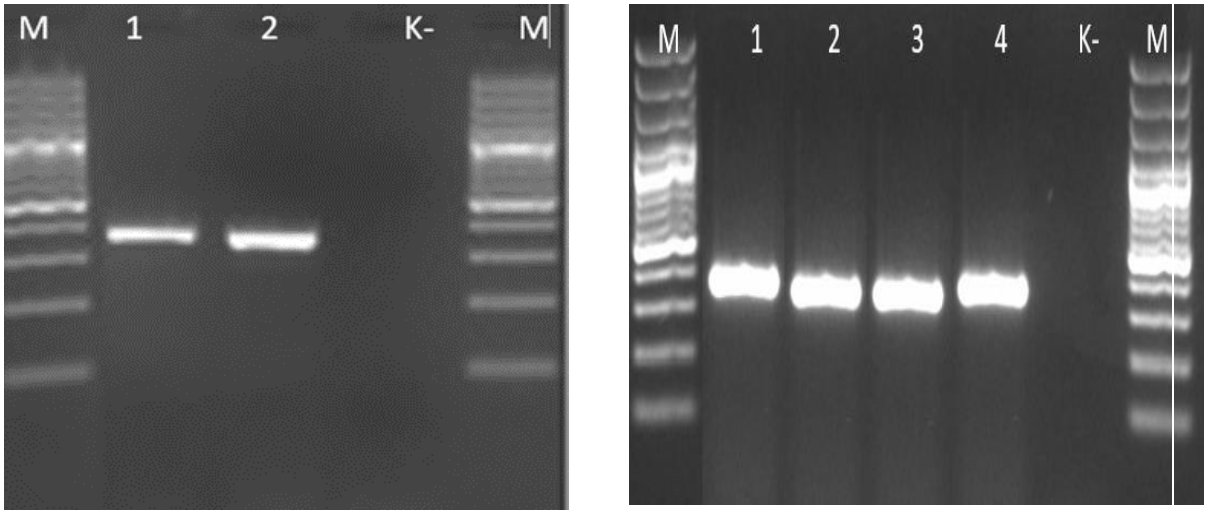



Figure 17. - Evaluation of the selectivity of a pair of primers dsn.1 F / R. for the diagnosis of *Ditylenchus destructor* from different regions: A. Ardebil province; B. Isfahan Province; C. Zanjan province; D. Hamedan province; M: marker, K-: negative control (Different result band thickness may be for different DNA concentration)

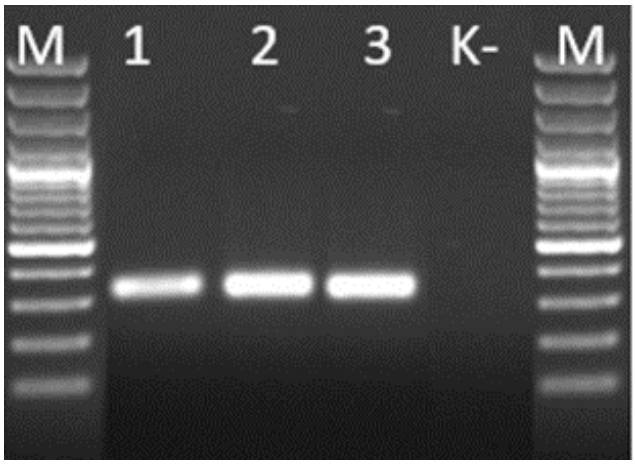



Figure 18. - Evaluation of the selectivity of the dsn.1F/R primer pair. for the diagnosis of *Ditylenchus destructor* from various regions: 1. Moscow region; 2. Nizhny Novgorod region; 3. Bryansk region; M: marker; K-: negative control (Different result band thickness may be for different DNA concentration)

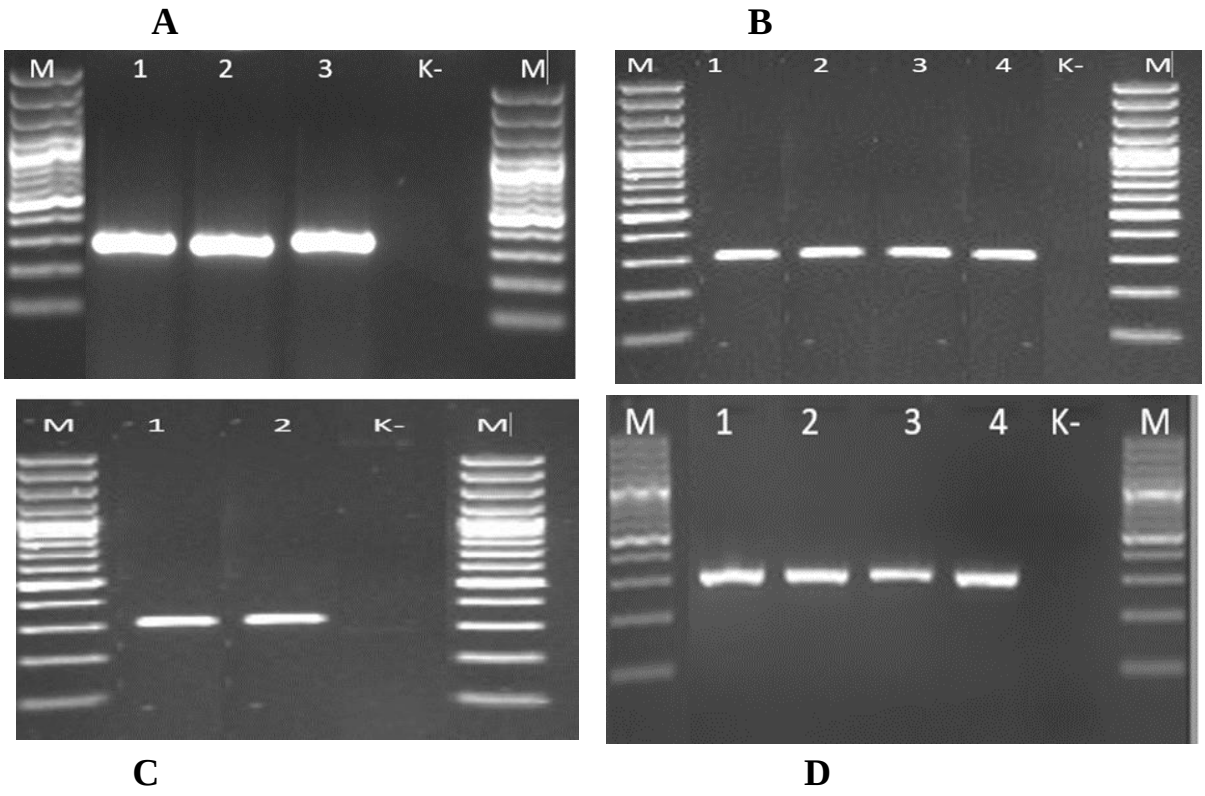



Figure 19. - Evaluation of the selectivity of a pair of primers dsn.2 F / R. for the diagnosis of *Ditylenchus destructor* from different regions: A. Ardebil province; B. Isfahan Province; C. Zanjan province; D. Hamedan province; M: marker, K-: negative control (Different result band thickness may be for different DNA concentration)

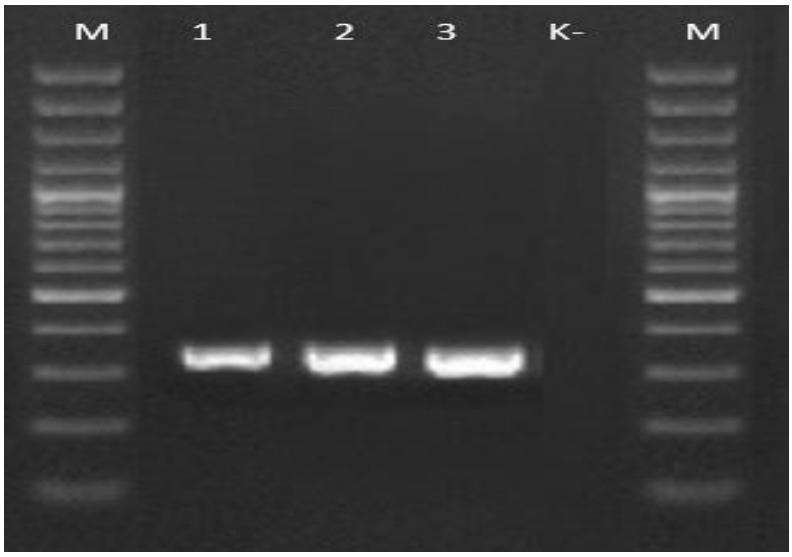



Figure 20. - Evaluation of the selectivity of the dsn.2F/R primer pair. for the diagnosis of *Ditylenchus destructor* from various regions: 1. Moscow region; 2. Nizhny Novgorod region; 3. Bryansk region; M: marker; K-: negative control (Different result band thickness may be for different DNA concentration)

All *D. destructor* accessions obtained from Iran and Russia were successfully amplified with primer pairs dsn.1 F/R and dsn.2 F/R. All samples showed one bright and clear target an expected-sized band: about 397 bp. for dsn.1 F/R and 330 bp. for dsn.2 F/R. Fragments were absent in the negative control. The results obtained indicate the effectiveness of the designed primers (Figures 17, 18, 19, and 20).

### 3.2.5.2. Test for primer specificity

The method's specificity resides in its ability to separate target species from closely related and morphologically similar species. DNA from the target species, *D. destructor*, as well as DNA from *D. gigas* and *D. dipsaci*, was used in this study. There are four iterations of the reaction. Figures 21 and 22 depict the findings.

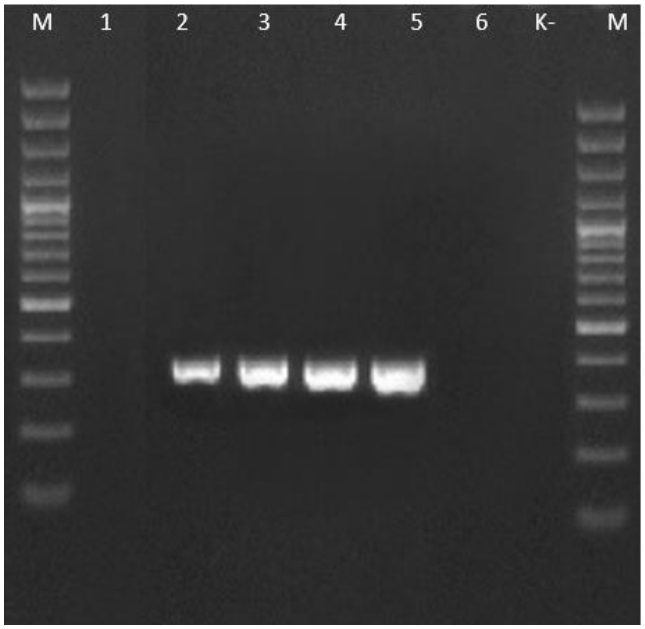



Figure 21. - Analyzing the specificity of the created pairs of primers for *Ditylenchus destructor*: 1. *D. dipsaci*; 2. Ardebil province; 3. Isfahan Province; 4. Zanjan province; 5. Hamadan province; 6. *D. gigas*; M: marker, K-: negative control (Different result band thickness may be for different DNA concentration)

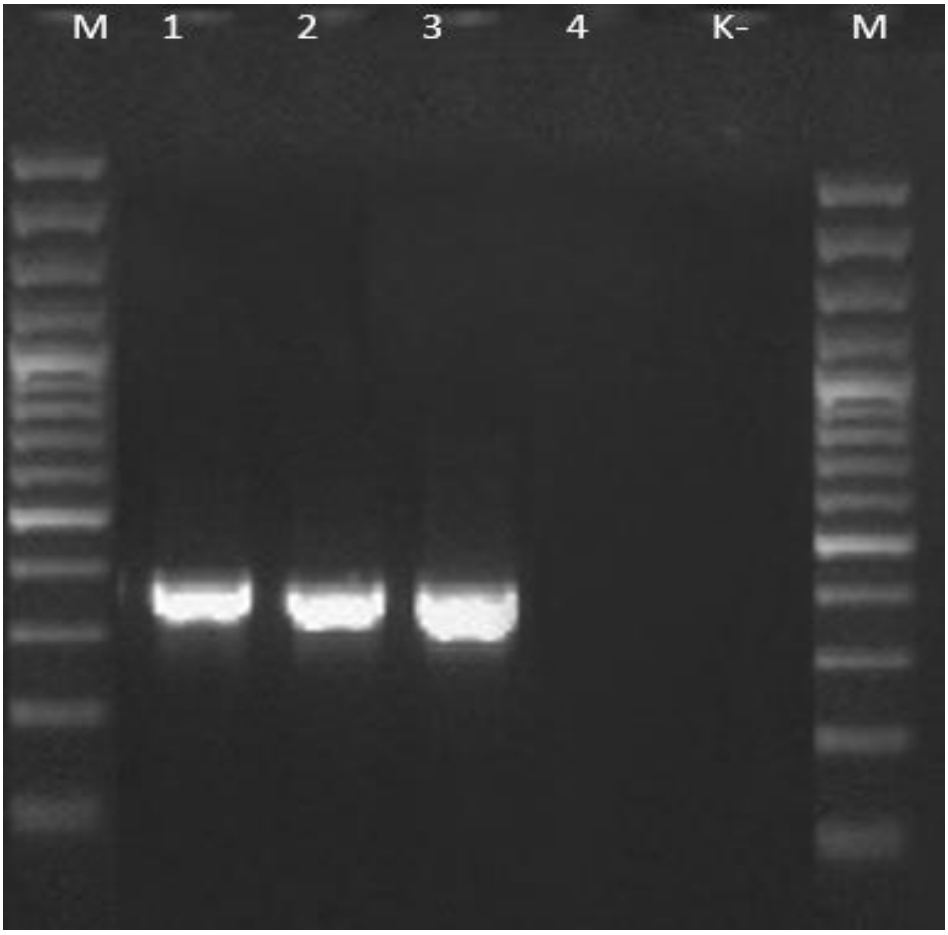



Figure 22. - Evaluation of the specificity of the generated primer pairs for *Ditylenchus destructor*; 1. Moscow region; 2. Nizhny Novgorod region; 3. Bryansk region; 4. *D. dipsaci*; K-: negative control; M: Marker (Different result band thickness may be for different DNA concentration)

### 3.2.5.3. Primer sensitivity test

The analytical sensitivity of PCR is the minimum amount of DNA from a target that can be reliably identified using this method. To assess the specificity of PCR on new examples, a series of dilutions of *D. destructor* DNA was prepared: approximately 1 ng, 5 ng, 50 ng, 100 ng. The reaction was carried out in 4 repetitions, in each of which similar results were obtained (Figure 23 and 24).

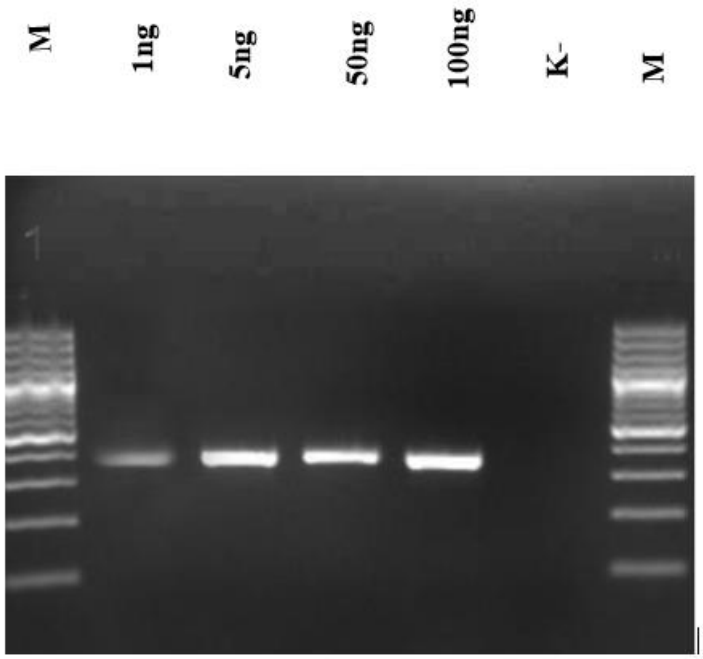



Figure 23. - Sensitivity evaluation of the dsn.1 F/R primer pair with different amounts of *Ditylenchus destructor* DNA. One of 4 similar repetitions. M: DNA marker, K-negative control

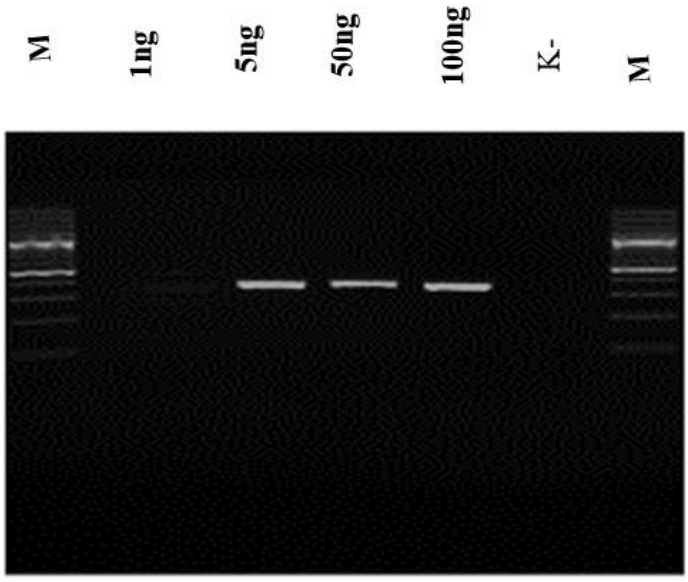



Figure 24. - Sensitivity evaluation of the dsn.2 F/R primer pair with different amounts of *Ditylenchus destructor* DNA. One of 4 similar repetitions. M: DNA marker, K-negative control

On electrophoresis through gels, PCR products run with the primer pairs dsn.1 F/R, and dsn.2 F/R and the corresponding template each produced a band of the expected size (397 and 330 bp, respectively). However, using the first pair of primers (dsn.1 F/R), the result was clearer and more visible, which may be a response to temperature changes and the number of G and C nucleotide bases The existence of repetitive components in ITS1 was linked to variations in the length of this rRNA fragment [121, 122]. In *D. destructor* ITS1, [102] discovered repetitive components of three, four, and eleven nucleotides. 5.8S was roughly 154 bp long, ITS2 was 207 bp long, and ITS1 ranged from 315 to 473 bp in length in *D. destructor* [119]. Also, the amount of *D. destructor* DNA affected the sensitivity of primers for dsn.1 F/R, cross-reaction products were observed for 100, 50.5 and 1 ng of *D. destructor* DNA, but were not observed for dsn.2 F/R at 100 ng.

Effective plant protection depends heavily on accurate identification of plant parasites harm the agroeconomy. Tandem repetitions found in ITS-rDNA sequences are easily observable genetic markers that accustomed to build Phylogenetic trees [92] are used to evaluate populations that are genetically linked and for diagnostic purposes. [93].

Despite the variation in nucleotides that was discovered for the *Ditylenchus* species, including interspecies variability [48, 118], it was still possible to locate a conserved region that would act as the foundation for forward and reverse primers that would be specific to each species [122].

With the help of species-specific PCR primers, the current PCR conditions enabled the precise identification of the studied species across all populations. The interpretation of the data from electrophoresis can be enhanced by using high resolution agarose gels. The ITS rRNA gene for *D. destructor* has large length variations that have never been observed for any other nematode, according to earlier research [119].

## Conclusion

-As a result of studying the morphological variability of *D. destructor* populations, it was shown that all the studied populations belong to the *D. destructor* species, despite the fact that it was reported that Iranian and Russian populations had minor differences in traits; In female's features: the longest Body length, Stylet length, and Pus length belong to Russian populations and the longest Tail length, Body length to Tail length ratio (C) and Pus/Anus-Vulva distance % belong to Iranian populations. In male's features: the longest Body length, Tail length, Stylet length, Bursa length and Body length to Tail length ratio (C) belong to Russian populations and the longest spicule length belongs to Iranian populations.

-NCBI GenBank has been supplemented with sequences (codes: MN122076, MN307126, MN307128, MN493767, MN658597, MN658599, MN658637, MN658638), which is a contribution to expanding the world's public knowledge about the genetic diversity of the *D. destructor* species.

- According to the phylogenetic tree, the sequences of our Iranian populations were very similar to the *D. destructor* samples founded in China and our Russian populations were similar to the other Russian *D. destructor* populations that identified previously by other researchers.

-To determine which species belong to the genus *Ditylenchus,* two new pairs of species-specific primers (dsn.1 F/R and dsn.2 F/R) are proposed. It was demonstrated that the unique the primer pairs dsn.1 F/R and dsn.2 F/R were highly effective at identifying *D. destructor*. The new primers dsn.1 F/R and dsn.2 F/specificity for *D. destructor* was demonstrated, as well as their lack of false-positive reactions for *Ditylenchus* species that are related to each other closely, *D. dipsaci* and *D. gigas*.

-It was found that the analytical sensitivity of the method with new primers is 1 ng of DNA for dsn.1 F/R and 5 ng of DNA for dsn.2 F/R. Like the first pair of primers, dsn.1 has a more specific identification.

## Bibliography

1. Гарипов Р. Р., Репина Е. М. СТЕРЖНЕВЫЕ ТЕРМОПЛАВКИЕ КЛЕИ И ИХ СВОЙСТВА // Ответственный редактор. – 2021. – С. 52.

2. Зиновьева С. В., Чижов В., Приданников М., Субботин С., Рысс А., Хусаинов Р. Фитопаразитические нематоды России // Товарищество научных изданий КМК. – 2012.

3. Chitwood D. J. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture–Agricultural Research Service // Pest Management Science: Formerly Pesticide Science. – 2003. – Т. 59, № 6-7. – С. 748-753.

4. Abad P., Gouzy J., Aury J.-M., Castagnone-Sereno P., Danchin E. G., Deleury E., Perfus-Barbeoch L., Anthouard V., Artiguenave F., Blok V. C. Genome sequence of the metazoan plant-parasitic nematode Meloidogyne incognita // Nature biotechnology. – 2008. – Т. 26, № 8. – С. 909-915.

5. Koenning S., Overstreet C., Noling J., Donald P., Becker J., Fortnum B. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994 // Journal of nematology. – 1999. – Т. 31, № 4S. – С. 587.

6. Brzeski M. Agricultural nematology //. – 1993.

7. Plowright R., Caubel G., Mizen K. Ditylenchus species // Plant resistance to parasitic nematodes. – 2002. – С. 107-139.

8. Maggenti A. R., Nickle W. Nemata: higher classification // Manual of agricultural nematology. – 1991. – С. 147-187.

9. Vanstone V. A., Russ M. H. Ability of weeds to host the root lesion nematodes Pratylenchus neglectus and P. thornei I. Grass weeds // Australasian Plant Pathology. – 2001. – Т. 30, № 3. – С. 245-250.

10. Siddiqi M. R. Studies on Tylencharhynchus spp.(Nematoda: Tylenchida) from India // Zeitschrlft fur Parasitenkunde. – 1961. – T. 21, № 1. – C. 46-64.

11. Hashemi K., Karegar A. Description of Ditylenchus paraparvus n. sp. from Iran with an updated list of Ditylenchus Filipjev, 1936 (Nematoda: Anguinidae) // Zootaxa. – 2019. – T. 4651, № 1. – C. zootaxa. 4651.1. 6-zootaxa. 4651.1. 6.

12. De Giorgi C., Sialer M. F., Di Vito M., Lamberti F. Identification of plant-parasitic nematodes by PCR amplification of DNA fragments 1 // EPPO bulletin. – 1994. – T. 24, № 2. – C. 447-451

13. Vovlas N., Troccoli A., Palomares-Rius J. E., De Luca F., Liébanas G., Landa B. B., Subbotin S. A., Castillo P. Ditylenchus gigas n. sp. parasitizing broad bean: a new stem nematode singled out from the Ditylenchus dipsaci species complex using a polyphasic approach with molecular phylogeny // Plant Pathology. – 2011. – T. 60, № 4. – C. 762-775.

14. Zhang S., Liu G., Janssen T., Zhang S., Xiao S., Li S., Couvreur M., Bert W. A new stem nematode associated with peanut pod rot in China: morphological and molecular characterization of Ditylenchus arachis n. sp.(Nematoda: Anguinidae) // Plant pathology. – 2014. – T. 63, № 5. – C. 1193-1206.

15. Abivardi C., Sharafeh M. The alfalfa stem nematode Ditylenchus dipsaci (Kühn 1857) Filipjev 1936 as an important threat for cultivation of alfalfa in Iran // Nematologia Mediterranea. – 1973.

16. Al-Zidgali T., Mani A., Al-Hinai M. Ditylenchus dipsaci, new nematode pest affecting garlic in Oman // FAO Plant Protection Bulletin. – 1994. – T. 42, № 3. – C. 154-155.

17. Plant parasitic nematodes in temperate agriculture. / Evans K., Trudgill D. L., Webster J. M.: CAB international, 1993.

18. Sturhan D. Über verbreitung, pathogenität und taxonomie der nematodengattung Tylenchorhynchus // Mitteilungen der Biologischen Anstalt für Land-u Forstwirtschaft. – 1966. – Т. 118. – С. 82-99.

19. Mazdosht G., Mazaheri Nasab H., Mohammadi M. R., Sediqe F. Evaluation of succeptibility and resistance of Hamedani Lucerne (Medicago Sativa L.) ecotypes to alfaalfa stem nematode Ditylenchus dipsaci (kuhn, 1857) Filipejev, 1936 in Hamedan and its biology //. – 2003.

20. Thorne G. Ditylenchus destructor n. sp. the potato rot nematode, and Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936, the teasel nematode (Nematoda: Tylenchidae) // Proceedings of the helminthological Society of Washington. – 1945. – Т. 12, № 2. – С. 27-34.

21. Tenuta M., Madani M., Briar S., Molina O., Gulden R., Subbotin S. A. Occurrence of Ditylenchus weischeri and not D. dipsaci in field pea harvest samples and Cirsium arvense in the Canadian Prairies // Journal of Nematology. – 2014. – Т. 46, № 4. – С. 376.

22. Swart A., * Mounport, D.**, Cadet P. Description of Ditylenchus clavicaudatus sp n from Senegal and notes on D triformis Hirschman & Sasser from South Africa (Tylenchida: Anguinidae) // African Plant Protection. – 1999. – Т. 5, № 2. – С. 111-117.

23. Esser R. Characterization of potato rot nematode, Ditylenchus destructor Thorne, 1945 (Tylenchidae) for regulatory purposes // Nematology Circular. – 1985. – Т. 124.

24. Гуськова Л., Метлицкий О., Данилов Л., Белоножко Г., Болотный А., Зорьева Т., Хрянина Р. Методические указания по проведению государственных испытаний нематицидов //. – 1983.

25. Švilponis E. Factors influencing the distribution and overwintering survival of the potato rot nematode (Ditylenchus destructor Thorne1945); Eesti Maaülikool, 2011.

26. Hockland S., Inserra R., Millar L., Lehman P. International plant health-putting legislation into practice // Plant nematology. – 2006. – С. 327-345.

27. Anderson J., Bentley S., Irwin J., Mackie J., Neate S., Pattemore J. Characterisation of Rhizoctonia solani isolates causing root canker of lucerne in Australia // Australasian Plant Pathology. – 2004. – Т. 33, № 2. – С. 241-247.

28. Decker H. Plant Nematodes and Their Control (Phytonematology)(Ed. NM Sveshnikova). Translated from Russian by USDA and NSF, Washington DC Amerind Publ // Co. Put. Ltd., New Delhi. – 1969.

29. Sturhan D., Hampel G. Plant-parasitic nematodes as prey of the bulb mite Rhizoglyphus echinopus (Acarina, Tyroglyphidae) // Anzieger fur Schadlingskunde, Pflanzenschutz, Umweltschutz. – 1977. – Т. 50. – С. 115-118.

30. Carta L., Handoo Z., Powers T., Miller S., Pérez-Zubiri R., Ramírez-Suárez A. Guidelines for isolation and identification of regulated nematodes of potato (Solanum tuberosum L.) in North America // Revista Mexicana de Fitopatología. – 2005. – Т. 23, № 2. – С. 211-222.

31. Moafi A., Rahgozar S., Pourfathollah A., Hariri M. Blood group antigens frequency: a comparative study in intermediate thalassemics versus healthy people in Isfahan //. – 2005.

32. Kheiri A. Plant parasitic nematodes (Tylenchida) from Iran // Biologisch Jaarboek Dodonaea. – 1972. – Т. 40. – С. 224-239.

33. Kheiri A., Barooti S., Karimipour H. Tylenchida associated with field crops in Tehran and central provinces of Iran // Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen). – 2002. – Т. 67, № 3. – С. 707-713.

34. Al-Hazimi H., Al-Khathlan H. Z. New diterpene and flavonoids from Pulicaria somalensis // J. King Saud Univ. Sci. – 1993. – Т. 5. – С. 141-146.

35. Viglierchio D. Stylet-bearing nemas and growth of ponderosa pine seedlings // Forest Science. – 1978. – Т. 24, № 2. – С. 222-227.

36. Foot M. A., Wood F. Potato rot nematode, Ditylenchus destructor (Nematoda: Tylenchidae), infecting hops in New Zealand // New Zealand journal of experimental agriculture. – 1982. – Т. 10, № 4. – С. 443-446.

37. Planer F. Above ground stem infection caused by Ditylenchus destructor // Nematologia. – 1972. – Т. 18, № 3.

38. Mahmoudi N., Pridannikov M., Zargar M., Naserzadeh Y., Limantceva L., Pakina E. Molecular diagnostics of Ditylenchus destructor based on the ITSrDNA from Iran and Russia Federation // Research on Crops. – 2020. – Т. 21, № 1. – С. 151-155.

39. Syrota Y. Y., Kharchenko V. O. Analysis of study comprehensiveness for nematode fauna of hydrophilic birds in Ukrainian Polissya // Annals of Parasitology. – 2015. – Т. 61, № 3.

40. Katalan-Gateva S., Konstantinova-Milkova M. Two species of parasitic nematodes on hop in Bulgaria. 1 // Godishnik na Sofiiskiya Universitet, Biologicheskie Fakultet, year 1973/74-1974/75, Kniga 1, Zoologiya. – 1975. – Т. 68. – С. 49-56.

41. Plant Nematodes and Their Control: Phytonematology. / Decker H.: Brill, 1989.

42. Xu Y.-M., Zhao Z.-Q. Longidoridae and Trichodoridae (Nematoda: Dorylaimida and Triplonchida) // Fauna of New Zealand. – 2019. – Т. 79.

43. Diano M. A., Dalan L., Singh P. R., Sumaya N. H. First report, morphological and molecular characterization of Caenorhabditis brenneri (Nematoda: Rhabditidae) isolated from the giant African land snail Achatina fulica (Gastropoda: Achatinidae) // Biologia. – 2022. – С. 1-10.

44. Yaghoubi A., Pourjam E., Ye W., Castillo P., Pedram M. Description and molecular phylogeny of Ditylenchus gilanicus n. sp.(Nematoda: Anguinidae) from northern forests of Iran // European Journal of Plant Pathology. – 2018. – Т. 152, № 3. – С. 735-746.

45. Maggenti A., Hart W. Carbamate and phosphate nematicidal granules, drenches and dips for the control of Ditylenchus destructor on bulbous iris, variety Wedgewood // Plant Disease Reporter. – 1975. – Т. 59, № 3. – С. 233-235.

46. Kim Y. H., Ohh S. H. Changes of Ditylenchus destructor of Ginseng // Korean J. Plant Pathol. – 1995. – Т. 11, № 1. – С. 39-46.

47. Huang W.-K., Peng D.-L., Zhang D.-S., Jiang H.-Y., Ding Z., Peng H., Long H.-B. Assessement of genetic variability in population of Ditylenchus destructor (Thorne 1945)(Tylenchida: Anguinidae) from China // Russian Journal of Nematology. – 2010. – Т. 18, № 1. – С. 19-30.

48. Golden A. M., O'Bannon J., Santo G., Finley A. Description and SEM observations of Meloidogyne chitwoodi n. sp.(Meloidogynidae), a root-knot nematode on potato in the Pacific Northwest // Journal of nematology. – 1980. – Т. 12, № 4. – С. 319.

49. Santo G., O'bannon J., Finley A., Golden A. Occurrence and host range of a new root-knot nematode (Meloidogyne chitwoodi) in the Pacific Northwest // Plant Disease. – 1980. – Т. 64, № 10. – С. 951-952.

50. Hooper D. Ditylenchus destructor // CIH descriptions of plant-parasitic nematodes, set. – 1973. – Т. 2. – С. 268-272.

51. Goodey J. Ditylenchus myceliophagus n. sp.(Nematoda: Tylenchidae) // Nematologica. – 1958. – Т. 3, № 2. – С. 91-96.

52. Andersson S. The potato rot nematode, Ditylenchus destructor Thorne, as a parasite in potatoes // The potato rot nematode, Ditylenchus destructor Thorne, as a parasite in potatoes. – 1971.

53. Ivaniuk V., Kalach V., Ilyashenka D., Yerchyk V., Vlasenko A. Ways of optimization of phytosanitary conditions in potato in Belarus // ZEMDIRBYSTE-AGRICULTURE. – 2008. – Т. 95, № 3. – С. 82-87.

54. Afonin A. Atlas of economic plants and pests in Russia and neighboring countries // http://www. agroatlas. ru. – 2006.

55. Giti M., Kashi L., Pedram M. Data of an Iranian population of L. proximus Sturhan & Argo, 1983, with taxonomic revision of L. israelensis Peneva, Orion, Shlevin, bar-Eyal & Brown, 1998 (Nematoda: Longidoridae) and proposal for a new synonymy // Journal of Nematology. – 2019. – T. 51.

56. Tanha Maafi Z., Kheiri A. Some species of plant parasitic nematodes of citrus in Hormozgan province // Proceedings of 9th Iranian Plant Protection Congress. Mashhad, Iran –, 1989. – C. 180.

57. Barooti S. The plant nematode fauna of cultivated soil from East-Azarbaijan, Ardabil and Moghan // Applied Entomology and Phytopathology. – 1997. – T. 66, № 1&2. – C. 32-35.

58. Wesemael W., Viaene N., Moens M. Root-knot nematodes (Meloidogyne spp.) in Europe // Nematology. – 2011. – T. 13, № 1. – C. 3-16.

59. Butorina N., Zinov'eva S., Kulinich O. Stem and leaf nematodes of plants // Applied nematology. Nauka, Moscow. – 2006. – C. 101-121.

60. Ding Z., Lin M. Identification of stem nematodes from sweet potatoes, potatoes and mint in China // ACTA Phytophylacica Sinica. – 1982. – T. 9, № 3. – C. 169-172.

61. Subbotin S. A., Madani M., Krall E., Sturhan D., Moens M. Molecular diagnostics, taxonomy, and phylogeny of the stem nematode Ditylenchus dipsaci species complex based on the sequences of the internal transcribed spacer-rDNA // Phytopathology. – 2005. – T. 95, № 11. – C. 1308-1315.

62. De D. Effect of temperature on the in vitro reproduction of Ditylenchus destructor isolated from groundnut // Revue Nématol. – 1990. – T. 13, № 2. – C. 171-174.

63. De Waele D., Coomans A. Occurrence and ecology of trichodorid nematodes in Belgium // Revue de nématologie. – 1991. – T. 14, № 1. – C. 127-132.

64. Diseases, pests and disorders of potatoes: a color handbook. / Wale S. J., Platt H. W., Cattlin N. D.: Elsevier, 2008.

65. Hague N. Perspectives in applied nematology // Nematology. – 2009. – T. 11, № 1. – C. 1-10.

66. Yigezu Wendimu G. Biology, Taxonomy, and Management of the Root-Knot Nematode (Meloidogyne incognita) in Sweet Potato // Advances in Agriculture. – 2021. – T. 2021.

67. Tenente R. C. Nematode problems of bulbs, with special reference to Ditylenchus dipsaci // Nematropica. – 1996. – T. 26, № 1. – C. 91-99.

68. Li J., Tian X., Perros A. P., Franssila S., Jokinen V. http://onlinelibrary. wiley. com/doi/10.1002/admi. 201400001/abstract //.

69. Thorne G. Principles of nematology // Soil Science. – 1962. – T. 93, № 1. – C. 70.

70. Wu L.-Y. Morphology of Ditylenchus destructor Thorne, 1945 (Nematoda: Tylenchidae), from a pure culture, with special reference to reproductive systems and esophageal glands // Canadian Journal of Zoology. – 1958. – T. 36, № 4. – C. 569-576.

71. Wu L. Further observations on the morphology of Ditylenchus destructor Thorne, 1945 (Nematoda: Tylenchidae) // Canadian Journal of Zoology. – 1960. – T. 38, № 1. – C. 47-49.

72. Evans A., Fisher J. The effect of environment on nematode morphometrics. Comparison of Ditylenchus myceliophagus and D. destructor // Nematologica. – 1970. – T. 16, № 1. – C. 113-122.

73. Wendt K., Swart A., Vrain T., Webster J. Ditylenchus africanus sp. n. from South Africa; a morphological and molecular characterization // Fundamental and Applied Nematology. – 1995. – T. 18, № 3. – C. 241-250.

74. Giblin-Davis R. M., Erteld C., Kanzaki N., Ye W., Zeng Y., Center B. J. Ditylenchus halictus n. sp.(Nematoda: Anguinidae), an associate of the sweat bee,

Halictus sexcinctus (Halictidae), from Germany // Nematology. – 2010. – T. 12, № 6. – C. 891-904.

75. Karani H. M., Eskandari A., Ghaderi R., Heydari R., Miraeez E. Morphological characterisation of a new and two known species of Ditylenchus Filipjev, 1936 (Nematoda: Anguinidae) from Iran // Zootaxa. – 2017. – T. 4216, № 4. – C. zootaxa. 4216.4. 4-zootaxa. 4216.4. 4.

76. Geraert E., Choi Y. Ditylenchus longicauda sp. n. a primitive ditylench // Revue Nématologie. – 1988. – T. 11. – C. 289-293.

77. Vovlas N., Troccoli A., Palomares-Rius J. E., De Luca F., Cantalapiedra-Navarrete C., Liébanas G., Landa B. B., Subbotin S. A., Castillo P. A new stem nematode, Ditylenchus oncogenus n. sp.(Nematoda: Tylenchida), parasitizing sowthistle from Adriatic coast dunes in southern Italy // Journal of Helminthology. – 2016. – T. 90, № 2. – C. 152-165.

78. Brzeski M. W. Review of the genus Ditylenchus Filipjev, 1936 (Nematoda: Anguinidae) // Revue de Nématologie. – 1991. – T. 14, № 1. – C. 9-59.

79. Mountain D., Wakefield N. H., Charles L. M., McGillivray L. A. The Invasive Species Compendium: information for the management of invasive species // INVASIVE APPLE SNAILS. – 2017. – C. 337.

80. Timper P. Utilization of biological control for managing plant-parasitic nematodes // Biological Control of Plant-Parasitic Nematodes:Springer, 2011. – C. 259-289.

81. Yakimenko V., Lebedev R. CALCULATION OF VIBRATIONAL-SPECTRUM OF DIMERIC STRUCTURES OF RHODANINE // Book CALCULATION OF VIBRATIONAL-SPECTRUM OF DIMERIC STRUCTURES OF RHODANINE / EditorIZVESTIA VUZOV FIZIKA REVOLUTION SQUARE 1, 634050 TOMSK, RUSSIA, 1973. – C. 138-141.

82. Jones P. D. Hemispheric surface air temperature variations: a reanalysis and an update to 1993 // Journal of Climate. – 1994. – Т. 7, № 11. – С. 1794-1802.

83. Watt B. A., Lebrun R. A. Soil effects of Beauveria bassiana on pupal populations of the Colorado potato beetle (Coleoptera: Chrysomelidae) // Environmental entomology. – 1984. – Т. 13, № 1. – С. 15-18.

84. Way M. Rice arthropod pests and their management in the United States // Rice. Origin, history, technology, and production. – 2003. – С. 437-456.

85. Kruus K., Wang W. K., Ching J., Wu J. Exoglucanase activities of the recombinant Clostridium thermocellum CelS, a major cellulosome component // Journal of bacteriology. – 1995. – Т. 177, № 6. – С. 1641-1644.

86. Kühnhold S., Saint-Cast P., Kafle B., Hofmann M., Colonna F., Zacharias M. High-temperature degradation in plasma-enhanced chemical vapor deposition Al2O3 surface passivation layers on crystalline silicon // Journal of Applied Physics. – 2014. – Т. 116, № 5. – С. 054507.

87. Abebe E., Mekete T., Thomas W. K. A critique of current methods in nematode taxonomy // African Journal of Biotechnology. – 2011. – Т. 10, № 3. – С. 312-323.

88. Blok V. C., Powers T. O. Biochemical and molecular identification // Root-knot nematodes. – 2009. – С. 98-118.

89. Carneiro R., de Oliveira Lima F. S., Correia V. R. Methods and tools currently used for the identification of plant parasitic nematodes // Nematology-Concepts, Diagnosis and Control. – 2017. – Т. 19.

90. Oliveira C. M. G. d., Monteiro A. R., Blok V. C. Morphological and molecular diagnostics for plant-parasitic nematodes: working together to get the identification done // Tropical Plant Pathology. – 2011. – Т. 36, № 2. – С. 65-73.

91. Powers T. Nematode molecular diagnostics: from bands to barcodes // Annu. Rev. Phytopathol. – 2004. – T. 42. – C. 367-383.

92. Jeszke A., Budziszewska M., Dobosz R., Stachowiak A., Protasewicz D., Wieczorek P., Obrępalska-Stęplowska A. A Comparative and Phylogenetic Study of the D itylenchus dipsaci, D itylenchus destructor and D itylenchus gigas Populations Occurring in P oland // Journal of Phytopathology. – 2014. – T. 162, № 1. – C. 61-67.

93. Nowaczyk K., Obrepalska-Steplowska A., Gawlak M., Throne J., Olejarski P., Nawrot J. Molecular techniques for detection of Tribolium confusum infestations in stored products // Journal of economic entomology. – 2009. – T. 102, № 4. – C. 1691-1695.

94. Chizhov V. N., Borisov B. A., Subbotin S. A. A new stem nematode, Ditylenchus weischeri sp. n.(Nematoda: Tylenchida), a parasite of Cirsium arvense (L.) Scop. in the central region of the non-chernozem zone of Russia // Russian Journal of Nematology. – 2010. – T. 18, № 2. – C. 95-102.

95. Maafi Z. T., Subbotin S., Moens M. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences // Nematology. – 2003. – T. 5, № 1. – C. 99-111.

96. Gutiérrez-Gutiérrez C., Palomares-Rius J. E., Cantalapiedra-Navarrete C., Landa B. B., Esmenjaud D., Castillo P. Molecular analysis and comparative morphology to resolve a complex of cryptic Xiphinema species // Zoologica Scripta. – 2010. – T. 39, № 5. – C. 483-498.

97. Vale J., Meredith T. Poisoning due to aliphatic, aromatic and chlorinated hydrocarbons // Poisoning Diagnosis and TreatmentSpringer, 1981. – C. 153-159.

98. Oliveira R. D., Santin Â. M., Seni D. J., Dietrich A., Salazar L. A., Subbotin S. A., Mundo-Ocampo M., Goldenberg R., Barreto R. W. Ditylenchus gallaeformans sp.

n.(Tylenchida: Anguinidae)–a neotropical nematode with biocontrol potential against weedy Melastomataceae // Nematology. – 2013. – Т. 15, № 2. – С. 179-196.

99. Vrain T., Wakarchuk D., Levesque A., Hamilton R. Intraspecific rDNA restriction fragment length polymorphism in the Xiphinema americanum group // Fundamental and applied Nematology. – 1992. – Т. 15, № 6. – С. 563-573.

100. Marek M., Zouhar M., Rysanek P., Havranek P. Analysis of ITS sequences of nuclear rDNA and development of a PCR-based assay for the rapid identification of the stem nematode Ditylenchus dipsaci (Nematoda: Anguinidae) in plant tissues // Helminthologia. – 2005. – Т. 42, № 2. – С. 49.

101. Marek M., Zouhar M., Douda O., Mazakova J., Rysanek P. Bioinformatics-assisted characterization of the ITS1-5· 8S-ITS2 segments of nuclear rRNA gene clusters, and its exploitation in molecular diagnostics of European crop-parasitic nematodes of the genus Ditylenchus // Plant Pathology. – 2010. – Т. 59, № 5. – С. 931-943.

102. Pethybridge S. J., Gorny A., Hoogland T., Jones L., Hay F., Smart C., Abawi G. Identification and characterization of Ditylenchus spp. populations from garlic in New York State, USA // Tropical Plant Pathology. – 2016. – Т. 41, № 3. – С. 193-197.

103. Plant nematology. / Perry R. N., Moens M.: CABI, 2006.

104. Archidona-Yuste A., Navas-Cortes J. A., Cantalapiedra-Navarrete C., Palomares-Rius J. E., Castillo P. Remarkable diversity and prevalence of dagger nematodes of the genus Xiphinema Cobb, 1913 (Nematoda: Longidoridae) in olives revealed by integrative approaches // PloS One. – 2016. – Т. 11, № 11. – С. e0165412.

105. Indarti S., Subandiyah S., Wibowo A., Ajri M. First record: a stem and bulb plant parasitic nematode at Garlic area centre Temanggung, Central Java, Indonesia with species reference to Ditylenchus dipsaci // Jurnal Perlindungan Tanaman Indonesia. – 2018. – Т. 22, № 2. – С. 233-237.

106. Arnheim N., Erlich H. Polymerase chain reaction strategy // Annual review of biochemistry. – 1992. – Т. 61, № 1. – С. 131-156.

107. Application of DNA analysis to nematode taxonomy. / Curran J., Nickle W.: Marcel Dekker, Inc., New York, 1991.

108. Curran J., Robinson M. Molecular aids to nematode diagnosis //. – 1993.

109. El-Shafie H. A. F. Tuta absoluta (Meyrick)(Lepidoptera: Gelechiidae): An Invasive Insect Pest Threatening the World Tomato Production // Invasive Species-Introduction Pathways, Economic Impact, and Possible Management OptionsIntechOpen, 2020.

110. Vrain T., McNamara D. Potential for identification of quarantine nematodes by PCR 1 // EPPO Bulletin. – 1994. – Т. 24, № 2. – С. 453-458.

111. Hyman B. Molecular systematics and population biology of phytonematodes: some unifying principles // Fundamental and applied nematology. – 1996. – Т. 19, № 4. – С. 309-313.

112. Perry R. N., Maule A. G., Gaugler R., Bilgrami A. Physiological and biochemical basis of behaviour // Nematode Behavior. New Jersey: CABI Publishing. – 2004. – С. 197-238.

113. McCuiston J. L., Hudson L. C., Subbotin S. A., Davis E. L., Warfield C. Y. Conventional and PCR detection of Aphelenchoides fragariae in diverse ornamental host plant species // Journal of Nematology. – 2007. – Т. 39, № 4. – С. 343.

114. Jeszke A., Dobosz R., Obrępalska-Stęplowska A. A fast and sensitive method for the simultaneous identification of three important nematode species of the genus Ditylenchus // Pest management science. – 2015. – Т. 71, № 2. – С. 243-249.

115. Coordinators N. R. Database resources of the national center for biotechnology information // Nucleic acids research. – 2016. – Т. 44, № Database issue. – С. D7.

116. Mahmoudi N., Nejad D. K., Shayanmehr F. A new primer set for amplification of ITS-rDNA in Ditylenchus destructor // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. – 2020. – Т. 15, № 2. – С. 150-158

117. Cahyati U. C. Identifikasi Molekuler Nematoda Aphelenchoides besseyi Christie melalui Perunutan Nukleotida dan Asam Amino Gen Mitochondrial Cytochrome Oxidase I (Mt-CO1) //.

118. Esquibet M., Grenier E., Plantard O., Andaloussi F. A., Caubel G. DNA polymorphism in the stem nematode Ditylenchus dipsaci: development of diagnostic markers for normal and giant races // Genome. – 2003. – Т. 46, № 6. – С. 1077-1083.

119. Subbotin S. A., Deimi A. M., Zheng J., Chizhov V. N. Length variation and repetitive sequences of internal transcribed spacer of ribosomal RNA gene, diagnostics and relationships of populations of potato rot nematode, Ditylenchus destructor Thorne, 1945 (Tylenchida: Anguinidae) // Nematology. – 2011. – Т. 13, № 7. – С. 773-785.

120. Subbotin S. A., Krall E. L., Riley I. T., Chizhov V. N., Staelens A., De Loose M., Moens M. Evolution of the gall-forming plant parasitic nematodes (Tylenchida: Anguinidae) and their relationships with hosts as inferred from Internal Transcribed Spacer sequences of nuclear ribosomal DNA // Molecular Phylogenetics and Evolution. – 2004. – Т. 30, № 1. – С. 226-235.

121. Шестеперов А. А., Перевертин К. А., Багров Р. А., Бутенко К. О. Клубневая нематода картофеля: биология и контроль // Картофель и овощи. – 2018. № 7. – С. 27-31.

122. Goodey J. B., Franklin M. T., Hooper D. J. T. Goodey's' The nematode parasites of plants catalogued under their hosts' // T. Goodey's' The nematode parasites of plants catalogued under their hosts'. – 1965. № 3rd ed.(rev.).

123. http://www.ncbi.nlm.nih.gov/genbank

# Apendix

Sequences from Iran:

**MN307126.1. *Ditylenchus destructor*. Iran**

CTCTCATATCTTCGTCTCCTCAACGTAATCTCATCTGAAGACTAA
TAGTAGTTGACATTTCTTCTTCCCACATTCACTCCTTACATCCATTT
TCACGAGCCACCTCCTGTAATGCCCTATTCTCGCGAGGTACAATAT
AACAGAATTAGCTACGCTAACAATTAGAGACTTACTTGGGTCTAT
AAACGTAGAGCTATCTCATTTGCTTTCAACCCTTAGCCCTACGTGC
CTTGTTTTACTTTAGGGAAAAAATTTTACTCACTAAGGACTCTCCC
CTCTTTGTTCTTTTTAATTTACCTCTTACTGTTCCTCCCAGCAAAGT
TAGGAAAGACCCCTCCCCACTCTTCCTTCGACAACATTTTTTCCCG
GGTCCGCCTTTCGTTTTTTTTCCCTCTTCTTCTTTCCTCTCTTTTCCT
TTCATTTTTTACAAGTACCCGTGAGGGAAAGTTGCAAAGCACTTTG
AAGAGAGAGTTAAAGAGGACGTGAAACCGATAAGATGGAAACGG
ATAGAGCCGACGTATCTGGCCTGTATTCAGCCGGGTGGCTGTCCAT
CGCTGGTTAGTCAGTCCTGTTTCTGGCTGGCTGGCTAGTGATTGGG
CAGTTTTACTGGTGCATTTGCAGGTGGTGTGCGCCGAAGCTCGCGC
TATGCTTTGGCTGGATCGTTGCTTTGAGGTCTCCCTTCGGGCGAGA
AACCAGAGCTTCAGGAAGGCTATGTGCTGTGCGAGTAGTAGTGCA
CGGTATTCGGTTGACCCGGTTGTAGGTTTTTGCTGGGGTTGCAGTC
GCATGCGACTGTGCCTTGGTGGGTTCTTGCAGCTGGTTAGACCCCG
TGACATTCTTCGGTGTAAAAGTCGGTCATCTCTCCGACCCGTCTTG
AAACACGGACCAAGGAGTTTAACGAGTGTGCGAGTCATTGGGTGT
GAAAACTCAAAGGCGCAATGAAAGTAAAGGCTTCGCTTGTCGAGC
TTATATGCGACCTCGGTGGTTTCGGCCATTGAGAGCAGCATAGCCC
CGTCCCGACTGCTTGCAGTGGGGCGGAGGAAGAGCATACTCGCTG
AGACCCGAAAGATGGTGAACTATGCCTGAGCAGGACGAAGCCAG

**MN.I.2. *Ditylenchus destructor*. Iran**

ACAAGTACCGTGAGGGAAAGTTGCAAAGCACTTTGAAGAGAGAGTTAAAG
AGGACGTGAAACCGATAAGATGGAAACGGATAGAGCCGACGTATCTGGCCTGT
ATTCAGCCGGGTGGCTGTCCATCGCTGGTTAGTCAGTCCTGTTTCTGGCTGGCT
GGCTAGTGATTGGGCAGTTTTACTGGTGCATTTGCAGGTGGTGTGCGCCGAAGC
TCGCGCTATGCTTTGGCTGGATCGTTGCTTTGAGGTCTCCCTTCGGGCGAGAAA
CCAGAGCTTCAGGAAGGCTATGTGCTGTGCGAGTAGTAGTGCACGGTATTCGG
TTGACCCGGTTGTAGGTTTTTGCTGGGGTTGCAGTCGCATGCGACTGTGCCTTG
GTGGGTTCTTGCAGCTGGTTAGACCCCGTGACATTCTTCGGTGTAAAAGTCGGT
CATCTCTCCGACCCGTCTTGAAACACGGACCAAGGAGTTTAACGAGTGTGCGA
GTCATTGGGTGTGAAAACTCAAAGGCGCAATGAAAGTAAAGGCTTCGCTTGTC
GAGCTTATATGCGACCTCGGTGGTTTCGGCCATTGAGAGCAGCATAGCCCCGTC
CCGACTGCTTGCAGTGGGGCGGAGGAAGAGCATACTCGCTGAGACCCGAAAGA
TGGTGAACTATGCCTGAGCAGGACGAAGCCAGAGGAAACTCTGGTGGAAGTCC
GGAGCGGTTCTGACGTGCAAATCGATCGTCTGACTTGGGTATAGGGACGAAAG
ATCAATCGAACCTTC

**MN.I.3. *Ditylenchus destructor*. Iran**

ACAAGTACCGTGAGGGAAAGTTGCAAAGCACTTTGAAGAGAGAGTTAA
AGAGGACGTGAAACCGATAAGATGGAAACGGATAGAGCCGACGTATCTG
GCCTGTATTCAGCCGGGTGGCTGTCCATCGCTGGTTAGTCAGTCCTGTTTC
TGGCTGGCTGGCTAGTGATTGGGCAGTTTTACTGGTGCATTTGCAGGTGGT
GTGCGCCGAAGCTCGCGCTATGCTTTGGCTGGATCGTTGCTTTGAGGTCTC
CCTTCGGGCGAGAAACCAGAGCTTCAGGAAGGCTATGTGCTGTGCGAGTA
GTAGTGCACGGTATTCGGTTGACCCGGTTGTAGGTTTTTGCTGGGGTTGCA
GTCGCATGCGACTGTGCCTTGGTGGGTTCTTGCAGCTGGTTAGACCCCGTG
ACATTCTTCGGTGTAAAAGTCGGTCATCTCTCCGACCCGTCTTGAAACACG
GACCAAGGAGTTTAACGAGTGTGCGAGTCATTGGGTGTGAAAACTCAAAG
GCGCAATGAAAGTAAAGGCTTCGCTTGTCGAGCTTATATGCGACCTCGGT
GGTTTCGGCCATTGAGAGCAGCATAGCCCCGTCCCGACTGCTTGCAGTGG
GGCGGAGGAAGAGCATACTCGCTGAGACCCGAAAGATGGTGAACTATGCC
TGAGCAGGACGAAGCCAGAGGAAACTCTGGTGGAAGTCCGGAGCGGTTCT
GACGTGCAAATCGATCGTCTGACTTGGGTATAGGGACGAAAGATCAATCG
AACCTTCTAGTAGCTGG

**MN307128.1.** ***Ditylenchus destructor*****. Iran**

ACTAATAGTAGTTGACATTTCTTCTTCCCACATTCACTCCTTACAT
CCATTTTCACGAGCCACCTCCTGTAATGCCCTATTCTCGCGAGGTAC
AATATAACAGAATTAGCTACGCTAACAATTAGAGACTTACTTGGGT
CTATAAACGTAGAGCTATCTCATTTGCTTTCAACCCTTAGCCCTACG
TGCCTTGTTTTACTTTAGGGAAAAAATTTTACTCACTAAGGACTCTC
CCCTCTTTGTTCTTTTTAATTTACCTCTTACTGTTCCTCCCAGCAAAG
TTAGGAAAGACCCCTCCCCACTCTTCCTTCGACAACATTTTTTCCCG
GGTCCGCCTTTCGTTTTTTTTCCCTCTTCTTCTTTCCTCTCTTTTCCTTT
CATTTTTTACAAGTACCCGTGAGGGAAAGTTGCAAAGCACTTTGAA
GAGAGAGTTAAAGAGGACGTGAAACCGATAAGATGGAAACGGATA
GAGCCGACGTATCTGGCCTGTATTCAGCCGGGTGGCTGTCCATCGCT
GGTTAGTCAGTCCTGTTTCTGGCTGGCTGGCTAGTGATTGGGCAGTT
TTACTGGTGCATTTGCAGGTGGTGTGCGCCGAAGCTCGCGCTATGCT
TTGGCTGGATCGTTGCTTTGAGGTCTCCCTTCGGGCGAGAAACCAGA
GCTTCAGGAAGGCTATGTGCTGTGCGAGTAGTAGTGCACGGTATTC
GGTTGACCCGGTTGTAGGTTTTTGCTGGGGTTGCAGTCGCATGCGAC
TGTGCCTTGGTGGGTTCTTGCAGCTGGTTAGACCCCGTGACATTCTT
CGGTGTAAAAGTCGGTCATCTCTCCGACCCGTCTTGAAACACGGAC
CAAGGAGTTTAACGAGTGTGCGAGTCATTGGGTGTGAAAACTCAAA
GGCGCAATGAAAGTAAAGGCTTCGCTTGTCGAGCTTATATGCGACC
TCGGTGGTTTCGGCCATTGAGAGCAGCATAGCCCCGTCCCGACTGCT
TGCAGTGGGGCGGAGGAAGAGCATACTCGCTGAGACCCGAAAGAT
GGTGAACTATGCCTGAGCAGGACGAAGCCAGAGGAAACTCTGGTGG
AAGTCCGGAGCGGTTCTGACGTGCAAATCGATCGT

**MN.B.2. *Ditylenchus destructor*. Iran**

ACAAGTACCGTGAGGGAAAGTTGCAAAGCACTTTGAAGAGAGAGTTAAAGA
GGACGTGAAACCGATAAGATGGAAACGGATAGAGCCGACGTATCTGGCCTGTA
TTCAGCCGGGTGGCTGTCCATCGCTGGTTAGTCAGTCCTGTTTCTGGCTGGCTGG
CTAGTGATTGGGCAGTTTTACTGGTGCATTTGCAGGTGGTGTGCGCCGAAGCTC
GCGCTATGCTTTGGCTGGATCGTTGCTTTGAGGTCTCCCTTCGGGCGAGAAACCA
GAGCTTCAGGAAGGCTATGTGCTGTGCGAGTAGTAGTGCACGGTATTCGGTTGA
CCCGGTTGTAGGTTTTTGCTGGGGTTGCAGTCGCATGCGACTGTGCCTTGGTGGG
TTCTTGCAGCTGGTTAGACCCCGTGACATTCTTCGGTGTAAAAGTCGGTCATCTC
TCCGACCCGTCTTGAAACACGGACCAAGGAGTTTAACGAGTGTGCGAGTCATTG
GGTGTGAAAACTCAAAGGCGCAATGAAAGTAAAGGCTTCGCTTGTCGAGCTTAT
ATGCGACCTCGGTGGTTTCGGCCATTGAGAGCAGCATAGCCCCGTCCCGACTGC
TTGCAGTGGGGCGGAGGAAGAGCATACTCGCTGAGACCCGAAAGATGGTGAAC
TATGCCTGAGCAGGACGAAGCCAGAGGAAACTCTGGTGGAAGTCCGGAGCGGT
TCTGACGTGCAAATCGATCGTCTGACTTGGGTATAGGGACGAAAGATCAATCGA

**MN.B.3. *Ditylenchus destructor*. Iran**

ACAAGTACCGTGAGGGAAAGTTGCAAAGCACTTTGAAGAGAGAGTTAAAGA
GGACGTGAAACCGATAAGATGGAAACGGATAGAGCCGACGTATCTGGCCTGTA
TTCAGCCGGGTGGCTGTCCATCGCTGGTTAGTCAGTCCTGTTTCTGGCTGGCTGG
CTAGTGATTGGGCAGTTTTACTGGTGCATTTGCAGGTGGTGTGCGCCGAAGCTC
GCGCTATGCTTTGGCTGGATCGTTGCTTTGAGGTCTCCCTTCGGGCGAGAAACC
AGAGCTTCAGGAAGGCTATGTGCTGTGCGAGTAGTAGTGCACGGTATTCGGTTG
ACCCGGTTGTAGGTTTTTGCTGGGGTTGCAGTCGCATGCGACTGTGCCTTGGTGG
GTTCTTGCAGCTGGTTAGACCCCGTGACATTCTTCGGTGTAAAAGTCGGTCATCT
CTCCGACCCGTCTTGAAACACGGACCAAGGAGTTTAACGAGTGTGCGAGTCATT
GGGTGTGAAAACTCAAAGGCGCAATGAAAGTAAAGGCTTCGCTTGTCGAGCTT
ATATGCGACCTCGGTGGTTTCGGCCATTGAGAGCAGCATAGCCCCGTCCCGACT
GCTTGCAGTGGGGCGGAGGAAGAGCATACTCGCTGAGACCCGAAAGATGGTGA
ACTATGCCTGAGCAGGACGAAGCCAGAGGAAACTCTGGTGGAAGTCCGGAGCG
GTTCTGACGTGCAAATCGATCGTCTGACTTGGGTATAGGGACGAAAGATCAATC

**MN493767.1. *Ditylenchus destructor*. Iran**

TGGGCTTGCACTTTGCGCTTGTGTTTGCTGGTGCGCTTGGGCCTGGCTAATT
TGTGGGCGAAAAACGGCTTTGTTGGCCTCTAAGTTTTCCTGAGCAGTTGTATG
CTTCTTTGTCCGTGGCTGTGATGAAGGAAAACGGTACGTGGTTTTCGTAATCG
CGAGAGTTAATGAGCACTGGCTTTGGTGCCGCCAACACAAAACCCCAATTTTA
CAAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGATCACTCGGCTCGTAG
ATCGATGAAGAACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGATATTTT
GAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTTATCCTTTGGCAC
GTCTGATTCAGGGTCGTAAATACAAAACCCCAAGCTAATGGTGGTGATATGAC
CTGTGCGGACCGCTGTCTCTTTGGCCTAGCACGTGTTTCTTGTGCAGCCTCTTG
GCCAATGTTGACATCGCTCTCACTCGAGAAAACGCTGTCCAGTGTTTGGTGAC
ATTGCTGTAAGTCCTAGCGATTCCTATGGACGTAAGGCTTTGAAGCCAAACGC
AGAGCAGTCGATTTTTCGACCTGAATCTGACGTGATTACCCGCTGAACTTAAG

**MN.C.2. *Ditylenchus destructor*. Iran**

GTGGCTGTAGGTGAACCTGCTGCCGGATCATTAACGATCATACCAATCCACTTTCA
GTGGTTATATTAGTCCTCAAAGGTGGCATGCTTCTGCCATGCAGGCACAGAGTAGTTG
TCCCGCTCTGTATTTGTACTTGCGCATTTGGGCTTGCACTTTGCGCTGTGTACTTGCGCT
ATGTACTTGCTCTGTGTACTTGCTCTGTGTACTTGCGCTGTGTACTTGCTTTAGAGCTTG
CATTTGTGCTTGCATTTGAGCTTGCATTTGAGCTTGCATTTGTGCTTGCATTTGCGCTTG
TGTTTGCTGGTGCGCTTGTGCCTGGCTAATTTGTGGGCGAAAAACGGCTTTGTTGGCCT
CTAAGTTTTCCTGAGCAGTTGTATGCTTCTTTGTCCGTGGCTGTGATGAAGGAAAACGG
TACGTGGTTTTCGTAATCGCGAGAGTTAATGAGCACTGGCTTTGGTGCCGCCAACACA
AAACCCCAATTTTACAAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGATCACTC
GGCTCGTAGATCGATGAAGAACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGAT
ATTTTGAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTTATCCTTTGGCACG
TCTGATTCAGGGTCGTAAATACAAAACCCCAAGCTAATGGTGGTGATATGACCTGTGC
GGACCGCTGTCTCTTTGGCCTAGCACGTGTTTCTTGTGCAGCCTCTTGGCCAATGTTGA
CATCGCTCTCACTCGAGAAAACGCTGTCCAGTGTTTGGTGACATTGCTGTAAGTCCTAG
CGATTCCTATGGACGTAAGGCTTTGAAGCCAAACGCAGAGCAGTCGATTTTTCGACCT
GAATCTGACGTGATTACCCGCTGAACTTAAG

**MN.C.3. *Ditylenchus destructor*. Iran**

GTTTCCGTAGGTGAACCTGCTGCCGGATCATTAACGATCATA
CCAATCCACTTTCAGTGGTTATATTAGTCCTCAAAGGTGGCATG
CTTCTGCCATGCAGGCACAGAGTAGTTGTCCCGCTCTGTATTTG
TACTTGCGCATTTGGGCTTGCACTTTGCGCTGTGTACTTGCGCT
ATGTACTTGCTCTGTGTACTTGCTCTGTGTACTTGCGCTGTGTAC
TTGCTTTAGAGCTTGCATTTGTGCTTGCATTTGAGCTTGCATTTG
AGCTTGCATTTGTGCTTGCATTTGCGCTTGTGTTTGCTGGTGCGC
TTGTGCCTGGCTAATTTGTGGGCGAAAAACGGCTTTGTTGGCCT
CTAAGTTTTCCTGAGCAGTTGTATGCTTCTTTGTCCGTGGCTGTG
ATGAAGGAAAACGGTACGTGGTTTTCGTAATCGCGAGAGTTAA
TGAGCACTGGCTTTGGTGCCGCCAACACAAAACCCCAATTTTAC
AAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGATCACTC
GGCTCGTAGATCGATGAAGAACGCAGCCAACTGCGATAATTAG
TGCGAACTGCAGATATTTTGAGCACTAAAGTTTCGAATGCACAT
TGCGCCATTGGATTTTATCCTTTGGCACGTCTGATTCAGGGTCG
TAAATACAAAACCCCAAGCTAATGGTGGTGATATGACCTGTGC
GGACCGCTGTCTCTTTGGCCTAGCACGTGTTTCTTGTGCAGCCT
CTTGGCCAATGTTGACATCGCTCTCACTCGAGAAAACGCTGTCC
AGTGTTTGGTGACATTGCTGTAAGTCCTAGCGATTCCTATGGAC
GTAAGGCTTTGAAGCCAAACGCAGAGCAGTCGATTTTTCGACC
TGAATCTGACGTGATTACCCGCTGAACTTAAG

**DNil.I.1. *Ditylenchus destructor*. Iran**

CCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGGACTGGGC
CATTTCGAGAAATTTGGGGATTGCTGATTAGCGATTCTTACGGATT
GCTTTTTGGTGAGAACCAATTTAATCGCAGTGGCCTGAACCGGGCA
AAAGTCGTAACAAGGTGGCTGTAGGTGAACCTGCTGCCGGATCAT
TAACGATCATACCAATCCACTTTCAGTGGTTATATTAGTCCTCAAA
GGTGGCATGCTTCTGCCATGCAGGCACAGAGTAGTTGTCCCGCTCT
GTATTTGTGCTTGCGCATTTGGGCTTGCACTTTGCGCTGTGTACTTG
CGCTATGTACTTGCTCTGTGTACTTGCTCTGTGTACTTGCGCTGTGT
ACTTGCTTTAGAGCTTGCATTTGTGCTTGCATTTGAGCTTGCATTTG
AGCTTGCATTTGTGCTTGCATTTGCGCTTGTGTTTGCTGGTGCGCTT
GTGCCTGGCTAATTTGTGGGCGAAAAACGGCTTTGTTGGCCTCTAA
GTTTTCCTGAGCAGTTGTATGCTTCTTTGTCCGTGGCTGTGATGAAG
GAAAACGGTACGTGGTTTTCGTAATCGCGAGAGTTAATGAGCACT
GGCTTTGGTGCCGCCAACACAAAACCCCAATTTTACAAATTTTTCA
AGAGAATATTTTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATC
GATGAAGAACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGAT
ATTTTGAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTT
ATCCTTTGGCACGTCTGATTCAGGGTCGTAAATACAAAACCCCAAG
CTAATGGTGGTGATATGACCTGTGCGGACCGCTGTCTCTTTGGCCT
AGCACGTGTTTCTTGTGCAGCCTCTTGGCCAATGTTGACATCGCTC
TCACTCGAGAAAACGCTGTCCAGTGTTTGGTGACATTGCTGTAAGT
CCTAGCGATTCCTATGGACGTAAGGCTTTGAAGCCAAACGCAGAG
CAGTCGATTTTTCGACCTGAATCTGACGTGATTACCCGCTGAACTT
AAGCATATCAGTAAGCGGAGGAAAAGAAACTAACAAGGATTC

**DNil.I.2. *Ditylenchus destructor*. Iran**

CGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGGACTGG
GCCATTTCGAGAAATTTGGGGATTGCTGATTAGCGATTCTTACGGAT
TGCTTTTTGGTGAGAACCAATTTAATCGCAGTGGCCTGAACCGGGCA
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AACGATCATACCAATCCACTTTCAGTGGTTATATTAGTCCTCAAAGG
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TTTGTACTTGCGCATTTGGGCTTGCACTTTGCGCTGTGTACTTGCGCT
ATGTACTTGCTCTGTGTACTTGCTCTGTGTACTTGCGCTGTGTACTTG
CTTTAGAGCTTGCATTTGTGCTTGCATTTGAGCTTGCATTTGAGCTTG
CATTTGTGCTTGCATTTGCGGTTGTGTTTGCTGGTGCGCTTGTGCCTG
GCTAATTTGTGGGCGAAAAACGGCTTTGTTGGCCTCTAAGTTTTCCT
GAGCAGTTGTATGCTTCTTTGTCCGTGGCTGTGATGAAGGAAAACG
GTACGTGGTTTTCGTAATCGCGAGAGTTAATGAGCACTGGCTTTGGT
GCCGCCAACACAAAACCCCAATTTTACAAATTTTTCAAGAGAATATT
TTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGATGAAGAACG
CAGCCAACTGCGATAATTAGTGCGAACTGCAGATATTTTGAGCACT
AAAGTTTCGAATGCACATTGCGCCATTGGATTTTATCCTTTGGCACG
TCTGATTCAGGGTCGTAAATACAAAACCCCAAGCTAATGGTGGTGA
TATGACCTGTGCGGACCGCTGTCTCTTTGGCCTAGCACGTGTTTCTT
GTGCAGCCTCTTGGCCAATGTTGACATCGCTCTCACTCGAGAAAACG
CTGTCCAGTGTTTGGTGACATTGCTGTAAGTCCTAGCGATTCCTATG
GACGTAAGGCTTTGAAGCCAAACGCAGAGCAGTCGATTTTTCGACC
TGAATCTGACGTGATTACCCGCTGAACTTAAGCATATCAGTAAGCG
GAGGAAAAGAAACTAACAAGGATTCCCTTAGTA

**DNil.I.3. Ditylenchus *destructor*. Iran**

TACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGG
ACTGGGCCATTTCGAGAAATTTGGGGATTGCTGATTAGCGATTC
TTACGGATTGCTTTTTGGTGAGAACCAATTTAATCGCAGTGGCC
TGAACCGGGCAAAAGTCGTAACAAGGTGGCTGTAGGTGAACCT
GCTGCCGGATCATTAACGATCATACCAATCCACTTTCAGTGGTT
ATATTAGTCCTCAAAGGTGGCATGCTTCTGCCATGCAGGCACAG
AGTAGTTGTCCCGCTCTGTATTTGTACTTGCGCATTTGGGCTTGC
ACTTTGCGCTATGTACTTGCGCTATGTACTTGCTCTGTGTACTTG
CTCTGTGTACTTGCGCTGTGTACTTGCTTTAGGGCTTGCATTAGT
GCTTGCATTAGAGCTTGCATTAGAGCTTGCATTTGTGCTTGCAT
TTGCGCTTGTGTTTGCTGGTGCGCTTGTGCCTGGCTAATTTGTGG
GCGAAAAACGGCTTTGTTGGCCTCTAAGTTTTCCTGAGCAGTTG
TATGCTTCTTTGTCCGTGGCTGTGATGAAGGAAAACGGTACGTG
GTTTTCGTAATCGCGAGAGTTAATGAGCACTGGCTTTGGTGCCG
CCAACACAAAACCCCAATTTTACAAATTTTTCAAGAGAATATTT
TTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGATGAAGAA
CGCAGCCAACTGCGATAATTAGTGCGAACTGCAGATATTTTGA
GCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTTATCCT
TTGGCACGTCTGATTCAGGGTCGTAAATACAAAACCCCAAGCT
AATGGTGGTGATATGACCTGTGCGGACCGCTGTCTCTTTGGCCT
AGCACGTGTTTCTTGTGCAGCCTCTTGGCCAATGTTGACATCGC
TCTCACTCGAGAAAACGCTGTCCAGTGTTTGGTGACATTGCTGT
AAGTCCTAGCGATTCCTATGGACGTAAGGCTTTGAAGCCAAAC
GCAGAGCAGTCGATTTTTCGACCTGAATCTGACGTGATTACCCG
CTGAACTTAAGCATATCAGTAAGCGGAGGAAAAGAAACTAACA
AGGATTCCCTTAG

**Sequences from Russia:**

**MN122076.1. *Ditylenchus destructor*. Russia**

CGCCTTTTGAATTACGTCCCTTGCCCCTTTGTAACACACCGTC
CCGTTACGCTGCCTGGGCACTTGGTCCCATCTTCCGAGAAATTTG
TGGGGGACTGGCTGATGTAGCGATTTCTGACAGATTGCGCTTTT
GGATGAGTAACCAATTTTAATCGCAGTGGCCTGAAGCCGGGCAA
AAGTTTCGTAACAAGGTTGGCTGTAGGTGAACCTGCTGCCGGGA
TCAGTTAACGATCATACCAATCCACTTTTCAGTGGTTATATTAGT
TCTTCAAAGGTGGCATGCTTCTGCCATGCAGGCACAGAGTAGGT
TGTTCCGCTCTGTATTTGTACTTGCGCATTTGGGCTTGCACTTGG
GCTGGCATTTGCGCTTTGTGTTTGCTGGTGCGCTTGTGCCTGGCT
AATTTGTGGGCGAAAAACGGCTTTGTTGGCCTCTAAGTTTTCCTG
AGCAGTTGTATGCTTCTTTGTCCGTGGCTGTGATGAAGGAAAAC
GGTACGTGGTTTTCGTAATCGCGAGAGTTAATGAGCACTGGCTT
TGGTGCCGCCAACACAAAACCCCAATTTTACAAATTTTTCAAGA
GAATATTTTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGA
TGAAGAACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGATA
TTTTGAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTT
ATCCTTTGGCACGTCTGATTCAGGGTCGTAAAATACAAAACCCC
AAGCTAATGGTGGTGATATGAACCTGTGCGGACCGGCTGTCTCT
TTGGCCTAGCACGTGTTTTCTTGTGCCAGCCTCTTGGCCAATGTT
GACATCCGCTCTTCCACTCGATGAAAACGCTTGTCCAATTGTGTT
TGGTGACATTTGGCTGTAAGTTCCTAGCGATTCCTATGGACGTAA
GGCTTTGAAGCCAAACGCAGAGCAGTCGATTTTTCGACCTGAAT
CTGACGTGATTACCCGCTGAACTTAAGCATATCAGT

**MN.1. R.2. *Ditylenchus destructor*. Russia**

TCCGTAGGTGAACCTGCTGCCGGATCATTAACGATCATACCAATCCACTTTCAG
TGGTTATATTAGTCCTCAAAGGTGGCATGCTTCTGCCATGCAGGCACAGAGTAGTT
GTCCCGCTCTGTATTTGTACTTGCGCTGTGTCGCTCTGTATTTGTACTTGCGCATTT
GCTGGTGCGCTTGTGCCTGGCTAATTTGTGGGCGAAAAACGGCTTTGTTGGCCTCT
AAGTTTTCCTGAGCAGTTGTATGCTTCTTTGTCCGTGGCTGTGATGAAGGAAAACG
GTACGTGGTTTTCGTAATCGCGAGAGTTAATGAGCACTGGCTTTGGTGCCGCCAAC
ACAAAACCCCAATTTTACAAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGA
TCACTCGGCTCGTAGATCGATGAAGAACGCAGCCAACTGCGATAATTAGTGCGAA
CTGCAGATATTTTGAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTTAT
CCTTTGGCACGTCTGATTCAGGGTCGTAAATACAAAACCCCAAGCTAATGGTGGTG
ATATGACCTGTGCGGACCGCTGTCTCTTTGGCCTAGCACGTGTTTCTTGTGCAGCCT
CTTGGCCAATGTTGACATCGCTCTCACTCGAGAAAACGCTGTCCAGTGTTTGGTGA
CATTGCTGTAAGTCCTAGCGATTCCTATGGACGTAAGGCTTTGAAGCCAAACGCAG
AGCAGTCGATTTTTCGACCTGAATCTGACGTGATTACCCGCTG

**MN.1. R.3. *Ditylenchus destructor*. Russia**

CATACCAATCCACTTTCAGTGGTTATATTAGTCCTCAAAGGTGGCATGCTTCTG
CCATGCAGGCACAGAGTAGTTGTCCCGCTCTGTATTTGTACTTGCGCATTTGGGCTT
GCACTTTGCGCTTGTGTTTGCTGGTGCGCTTGTGCCTGGCTAATTTGTGGGCGAAA
AACGGCTTTGTTGGCCTCTAAGTTTTCCTGAGCAGTTGTATGCTTCTTTGTCCGTGG
CTGTGATGAAGGAAAACGGTACGTGGTTTTCGTAATCGCGAGAGTTAATGAGCAC
TGGCTTTGGTGCCGCCAACACAAAACCCCAATTTTACAAATTTTTCAAGAGAATAT
TTTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGATGAAGAACGCAGCCAAC
TGCGATAATTAGTGCGAACTGCAGATATTTTGAGCACTAAAGTTTCGAATGCACAT
TGCGCCATTGGATTTTATCCTTTGGCACGTCTGATTCAGGGTCGTAAATACAAAAC
CCCAAGCTAATGGTGGTGATATGACCTGTGCGGACCGCTGTCTCTTTGGCCTAGCA
CGTGTTTCTTGTGCAGCCTCTTGGCCAATGTTGACATCGCTCTCACTCGAGAAAAC
GCTGTCCAGTGTTTGGTGACATTGCTGTAAGTCCTAGCGATTCCTATGGACGTAAG
GCTTTGAAGCCAAACGCAGAGCAGTCGATTTTTCGACCT

**MN658637.1. *Ditylenchus destructor*. Russia**

GAGAGTTAATGTAGCACTGGCTTTGGTGCCGGGGCCAACACAAAACCC
CAATTTTACAAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGATCACT
CGGCTCGTAGATCGATGAAGAACGCAGCCAACTGCGATAATTAGTGCGAA
CTGCAGATATTTTGAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGAT
TTTATCCTTTGGCACGTCTGATTCAGGGTCGTAAAATACAAAACCCCACCA
GCTAATGGTGGTGATATGAACCTGTGCGGACCGGCTGTCTCTTTGGCCTAG
CACGTGTTTTCTTGTGCCAGCCTCTTGGCCAATGTTGACATCCGCTCTTCCA
CTCGATAAAACGCTTGTCCAATTGTGTTTGGTGACATTTGGCTGTAAGTTCC
TAGCGATTCCTATGGACGTAAGGCTTTGAAGCCAAACGCAGAGCAGTCGA
TTTTTCGACCTGAATCTGACGTGATTCTCGCAGACAGACTACGT

**MN.2. R.2. *Ditylenchus destructor*. Russia**

GTACCCGTGGTCTTGTGAATGGGGCCAACACAAAACCCCAATT
TTACAAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGATCAC
TCGGCTCGTAGATCGATGAAGAACGCAGCCAACTGCGATAATTA
GTGCGAACTGCAGATATTTTGAGCACTAAAGTTTCGAATGCACAT
TGCGCCATTGGATTTTATCCTTTGGCACGTCTGATTCAGGGTCGTA
AAATACAAAACCCCACCAGCTAATGGTGGTGATATGAACCTGTG
CGGACCGGCTGTCTCTTTGGCCTAGCACGTGTTTTCTTGTGCCAGC
CTCTTGGCCAATGTTGACATCCGCTCTTCCACTCGATAAAACGCTT
GTCCAATTGTGTTTGGTGACATTTGGCTGTAAGTTCCTAGCGATTC
CTATGGACGTAAGGCTTTGAAGCCAAACGCAGAGCAGTCGATTTT
TCGACCTGAATCTGACGTGATTCTCGCAGACAGACTACGTATGGC
TTCCTTTTTTGGAACCCCCGGGTGGGGGGGTTAATTCCCAACCT

**MN.2. R.3. *Ditylenchus destructor*. Russia**

GCGCTTTGTGTTTGCTGGTGCGCTTGTGCCTGGCTAATTTGTGGGCGAAAA
ACGGCTTTGTTGGCCTCTAAGTTTTCCTGAGCAGTTGTATGCTTCTTTGTCCGT
GGCTGTGATGAAGGAAAACGGTACGTGGTTTTCGTAATCGCGAGAGTTAATG
AGCACTGGCTTTGGTGCCGCCAACACAAAACCCCAATTTTACAAATTTTTCAA
GAGAATATTTTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGATGAAGA
ACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGATATTTTGAGCACTAAA
GTTTCGAATGCACATTGCGCCATTGGATTTTATCCTTTGGCACGTCTGATTCA
GGGTCGTAAAATACAAAACCCCAAGCTAATGGTGGTGATATGAACCTGTGCG
GACCGGCTGTCTCTTTGGCCTAGCACGTGTTTTCTTGTGCCAGCCTCTTGGCC
AATGTTGACATCCGCTCTTCCACTCGATGAAAACGCTTGTCCAATTGTGTTTG
GTGACATTTGGCTGTAAGTTCCTAGCGATTCCTATGGACGTAAGGCTTTGAAG
CCAAACGCAGAGCAGTCGATTTTTCGACCTG

**MN658638.1. *Ditylenchus destructor*. Russia**

GCGCTTTGTGTTTGCTGGTGCGCTTGTGCCTGGCTAATTTGTGGGCGAAAAACG
GCTTTGTTGGCCTCTAAGTTTTCCTGAGCAGTTGTATGCTTCTTTGTCCGTGGCT
GTGATGAAGGAAAACGGTACGTGGTTTTCGTAATCGCGAGAGTTAATGAGCAC
TGGCTTTGGTGCCGCCAACACAAAACCCCAATTTTACAAATTTTTCAAGAGAA
TATTTTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGATGAAGAACGCAG
CCAACTGCGATAATTAGTGCGAACTGCAGATATTTTGAGCACTAAAGTTTCGA
ATGCACATTGCGCCATTGGATTTTATCCTTTGGCACGTCTGATTCAGGGTCGTA
AAATACAAAACCCCAAGCTAATGGTGGTGATATGAACCTGTGCGGACCGGCTG
TCTCTTTGGCCTAGCACGTGTTTTCTTGTGCCAGCCTCTTGGCCAATGTTGACAT
CCGCTCTTCCACTCGATGAAAACGCTTGTCCAATTGTGTTTGGTGACATTTGGC
TGTAAGTTCCTAGCGATTCCTATGGACGTAAGGCTTTGAAGCCAAACGCAGAG
CAGTCGATTTTTCGACCTGAATCTGACGTGATT

**MN. 3. R.2. *Ditylenchus destructor*. Russia**

ACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTGCCCGGGA
CTGGGCCATTTCGAGAAATTTGGGGATTGCTGATTAGCGATTCT
TACGGATTGCTTTTTGGTGAGAACCAATTTAATCGCAGTGGCCT
GAACCGGGCAAAAGTCGTAACAAGGTGGCTGTAGGTGAACCTG
CTGCCGGATCATTAACGATCATACCAATCCACTTTCAGTGGTTA
TATTAGTCCTCAAAGGTGGCATGCTTCTGCCATGCAGGCACAG
AGTAGTTGTCCCGCTCTGTATTTGTGCTTGCGCATTTGGGCTTG
CACTTTGCGCTGTGTACTTGCGCTATGTACTTGCTCTGTGTACTT
GCTCTGTGTACTTGCGCTGTGTACTTGCTTTAGAGCTTGCATTTG
TGCTTGCATTTGAGCTTGCATTTGAGCTTGCATTTGTGCTTGCAT
TTGCGCTTGTGTTTGCTGGTGCGCTTGTGCCTGGCTAATTTGTG
GGCGAAAAACGGCTTTGTTGGCCTCTAAGTTTTCCTGAGCAGTT
GTATGCTTCTTTGTCCGTGGCTGTGATGAAGGAAAACGGTACGT
GGTTTTCGTAATCGCGAGAGTTAATGAGCACTGGCTTTGGTGCC
GCCAACACAAAACCCCAATTTTACAAATTTTTCAAGAGAATATT
TTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGATGAAGA
ACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGATATTTTG
AGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTTATCC
TTTGGCACGTCTGATTCAGGGTCGTAAATACAAAACCCCAAGC
TAATGGTGGTGATATGACCTGTGCGGACCGCTGTCTCTTTGGCC
TAGCACGTGTTTCTTGTGCAGCCTCTTGGCCAATGTTGACATCG
CTCTCACTCGAGAAAACGCTGTCCAGTGTTTGGTGACATTGCTG
TAAGTCCTAGCGATTCCTATGGACGTAAGGCTTTGAAGCCAAA
CGCAGAGCAGTCGATTTTTCGACCTGAATCTGACGTGATTACCC
GCTGAACTTAAGCATATCAGTAAGCGGAGGAAAAGAAACTAAC
AAGGATTCCCTTAGTAACGGCGAGT

**MN.3. R.3. *Ditylenchus destructor*. Russia**

GGCTGTAGGTGAACCTGCTGCCGGATCATTAACGATCATACCAATCCACTTT
CAGTGGTTATATTAGTCCTCAAAGGTGGCATGCTTCTGCCATGCAGGCACAGAG
TAGTTGTCCCGCTCTGTATTTGTACTTGCGCATTTGGGCTTGCACTTTGCGCTGTG
TACTTGCGCTATGTACTTGCTCTGTGTACTTGCTCTGTGTACTTGCGCTGTGTACT
TGCTTTAGAGCTTGCATTTGTGCTTGCATTTGAGCTTGCATTTGAGCTTGCATTTG
TGCTTGCATTTGCGCTTGTGTTTGCTGGTGCGCTTGTGCCTGGCTAATTTGTGGG
CGAAAAACGGCTTTGTTGGCCTCTAAGTTTTCCTGAGCAGTTGTATGCTTCTTTG
TCCGTGGCTGTGATGAAGGAAAACGGTACGTGGTTTTCGTAATCGCGAGAGTTA
ATGAGCACTGGCTTTGGTGCCGCCAACACAAAACCCCAATTTTACAAATTTTTC
AAGAGAATATTTTTAGTCTTATCGGTGGATCACTCGGCTCGTAGATCGATGAAG
AACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGATATTTTGAGCACTAAAG
TTTCGAATGCACATTGCGCCATTGGATTTTATCCTTTGGCACGTCTGATTCAGGG
TCGTAAATACAAAACCCCAAGCTAATGGTGGTGATATGACCTGTGCGGACCGCT
GTCTCTTTGGCCTAGCACGTGTTTCTTGTGCAGCCTCTTGGCCAATGTTGACATC
GCTCTCACTCGAGAAAACGCTGTCCAGTGTTTGGTGACATTGCTGTAAGTCCTA
GCGATTCCTATGGACGTAAGGCTTTGAAGCCAAACGCAGAGCAGTCGATTTTTC

**MN658597. *Ditylenchus destructor*. Russia**

TTGGAGGCCCCCGGTACCCGTGGTCTTGTGAATGGGGCCAACACAAAACCCCAA
TTTTACAAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGATCACTCGGCTC
GTAGATCGATGAAGAACGCAGCCAACTGCGATAATTAGTGCGAACTGCAGATA
TTTTGAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTTATCCTTTGGC
ACGTCTGATTCAGGGTCGTAAAATACAAAACCCCACCAGCTAATGGTGGTGATA
TGAACCTGTGCGGACCGGCTGTCTCTTTGGCCTAGCACGTGTTTTCTTGTGCCAG
CCTCTTGGCCAATGTTGACATCCGCTCTTCCACTCGATAAAACGCTTGTCCAATT
GTGTTTGGTGACATTTGGCTGTAAGTTCCTAGCGATTCCTATGGACGTAAGGCTT
TGAAGCCAAACGCAGAGCAGTCGATTTTTCGACCTGAATCTGACGTGATTCTCG
CAGACAGACTACGTATGGCTTCCTTTTTTGGAACCCCCGGGTGGGGGGGTTAAT
TCCCAACCT

**MN658599. *Ditylenchus destructor*. Russia**

CGCGAGAGTTAATGTAGCACTGGCTTTGGTGCCGGGGCCAACACAAAACCCC
AATTTTACAAATTTTTCAAGAGAATATTTTTAGTCTTATCGGTGGATCACTCGG
CTCGTAGATCGATGAAGAACGCAGCCAACTGCGATAATTAGTGCGAACTGCA
GATATTTTGAGCACTAAAGTTTCGAATGCACATTGCGCCATTGGATTTTATCCT
TTGGCACGTCTGATTCAGGGTCGTAAAATACAAAACCCCACCAGCTAATGGTG
GTGATATGAACCTGTGCGGACCGGCTGTCTCTTTGGCCTAGCACGTGTTTTCTT
GTGCCAGCCTCTTGGCCAATGTTGACATCCGCTCTTCCACTCGATAAAACGCTT
GTCCAATTGTGTTTGGTGACATTTGGCTGTAAGTTCCTAGCGATTCCTATGGAC
GTAAGGCTTTGAAGCCAAACGCAGAGCAGTCGATTTTTCGACCTGAATCTGAC
GTGATTCTCGCAGACAGACTACGTATG