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FIRST REPORT OF *Robustodorus subtenuis* ON GARLIC PLANTS IN POLAND

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ABSTRACT

During the observation of a garlic plantation, a sample consisting of plants with symptoms of growth inhibition and the soil surrounding the roots was collected. Phytophagous nematodes were isolated from the soil sample as well as the garlic plant shoots, with an average density of 75 individuals per 100 mL. Microscope slides were prepared from adult individuals and subjected to morphological and morphometric analysis, as well as DNA examination. It was demonstrated that the examined nematodes belong to the species *Robustodorus subtenuis.* Both the female and male nematodes differed from individuals previously found in the cultivation of *Narcissus* and *Gladiolus*. On average, *R. subtenuis* females have shorter bodies and longer tails, with the averages calculated for the nematode population from the soil being slightly lower than the average values for nematodes from the garlic shoots. Additionally, the females found in the soil had a shorter distance from the lip region to the vulva and a shorter post-vulval sac, the length of which constituted 32 to 45% of the distance from the vulva to the anus. On average, the male bodies were shorter and had longer tails. They were also wider at the level of the anus. The average body length of males isolated from the soil, like that of the females, was lower than the average for individuals isolated from the garlic shoots. Variations in the influence of the host plant on the morphometry of the parasitic nematodes were observed. This is the first report of *R. subtenuis* colonizing garlic plants in Poland.

Keywords: foliar nematodes, *Allium sativum*, morphological characteristics, molecular identification

INTRODUCTION

Plant-parasitic nematodes are primarily known as organisms that feed either inside or on the outside of roots. These organisms also feed on leaves, stems, and their modified underground parts, such as tubers and bulbs, which can lead to a deterioration in the plant's physiological condition and even complete dieback [Handoo et al. 2020]. Some species can enter seeds and spread through this route. The symptoms of nem-

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atode feeding on aboveground parts of plants are typically leaf distortions, thickening of stems at the base, or stunting of plants [Green and Sime 1979].

The group known as leaf nematodes includes species such as *Litylenchus crenatae* Kanzaki, 2019 and *Litylenchus crenatae mccannii* Carta et al. 2020, as well as species from the genera *Ditylenchus* Filipjev, 1936 and *Aphelenchoides* Fisher, 1894. L. *crenatae* and L. *crenatae mccannii* are known from the northeastern regions of North America and adjacent areas in Ontario (Canada), where they are recognized as pests of trees from the genus *Fagus* L. and as damaging to *Coprosma repens* A. Rich shrubs [Kanzaki et al. 2019, Burke et al. 2020, Carta et al. 2020, Marra and La Mondia 2020, Reed et al. 2020, Kantor et al. 2022a, b, Vieira et al. 2023].

Among the species of the genus *Ditylenchus*, the economically significant species include *D. africanus* Wendt et al. 1995 and *D. arachis* Zhang et al. 2014, which are pests of *Arachis hypogaea* L.; *D. destructor* Thorne, 1945, a parasite of *Solanum tuberosum* ssp. *tuberosum* L. and numerous ornamental plant species; *D. dipsaci* (Kühn, 1857) Filipjev, 1936, a pest of onions (*Allium cepa* L.), garlic (*Allium sativum* L.), ornamental bulb plants, strawberries, and some perennials; and *Ditylenchus gigas* Vovlas et al. 2011, which feeds on *Vicia faba* L. [Kühn 1857, Thorne 1945, Wendt et al. 1995, Vovlas et al. 2011, Zhang et al. 2014, CABI 2024].

Among the nematodes of the genus *Aphelenchoides*, the most significant are *A. besseyi* Christie 1942, a pest of rice crops; *A. fragariae* (Ritzema Bos, 1890) Christie, 1932, which causes damage to strawberry plantations; and *A. rhitzemabosi* (Schwarz, 1911), Steiner and Buhrer 1932, a pest of strawberries and ornamental plants [Knight et al. 1997, Kohl 2011, Sánchez-Monge et al. 2015]. *A. blastophthorus* Franklin, 1952 was isolated from the aboveground parts of strawberry plants [Consoli et al. 2019]. Among the species of *Aphelenchoides*, *A. subtenuis* Cobb, 1926 is also significant for cultivated plant species. Following morphological analysis and phylogeny of the genus *Robustodorus* Andrássy, 2007, it was reclassified into this genus [Andrássy 2007, Kanzaki et al. 2018, Hodda 2022].

Robustodorus subtenuis (Cobb, 1926) Kanzaki et al. 2018 is a polyphagous species found on both monocotyledonous and dicotyledonous plants representing several botanical families, including *Fabaceae* Lindl., *Paeoniaceae* L., *Polemoniaceae* Juss., *Amaryllidaceae* J. St.-Hil., *Iridaceae* Juss., and *Liliaceae* Juss [Rathor and Tiwari 2016, Kanzaki et al. 2018]. The nematode was described from the bulbs and leaves of *Narcissus* L. [Goodey 1935] and has been repeatedly isolated from the tissues of this plant [Mor and Spiegel 1993, Knight et al. 1997, van Leeuwen and Trompert 2011, CABI 2024]. *R. subtenuis* has also been found in crocus bulbs, *Dahlia coccinea* Cav. and *Gladiolus communis* L. plants [Gandarilla Basterrechea and Fernández Gonzálvez 2002, Deimi et al. 2008], *Pandanus* sp. leaves [Gandarilla Basterrechea 2003], *Croccus* L. and *Tulipa* L. [van Leeuwen and Trompert 2011], *Iris* Tourn. ex L. [Sánchez-Monge et al. 2015], and rice [Stoyanov 1979]. From samples of capim-marandu grass exhibiting symptoms of growth inhibition and dieback, *R. subtenuis* was isolated in numbers ranging from 74 to 124 individuals per 100 mL of soil and 10 g of roots [Sharma et al. 2001].

Among leaf nematodes in Poland, *A. fragariae* and *A. rhitzemabosi* have been found, causing damage to strawberry plantations [Szczygieł 1967] and chrysanthemums [Baranowski 1976]. They also occur on several species of perennials and ornamental shrubs grown in nurseries [Chałańska et al. 2013]. *A. rhitzemabosi* was also isolated from leaf buds of *Ribes nigrum* L. [Chałańska et al. 2014] and from seeds of scarlet sage *Salvia splendens* -Sellow ex Roemer et Schultes- [Chałańska et al. 2011].

During the inspection of the garlic plantation, plants with symptoms of growth inhibition and yellowed leaves were found.

Since these symptoms were not specific to nematode infection, laboratory analysis of the plant material and the soil adhering to the roots was necessary to check for the presence of parasitic nematodes. The aim of the study was to demonstrate the presence of plant-parasitic nematodes that could have contributed to the symptoms observed on garlic plants.

MATERIAL AND METHODS

Collection of material for research

Garlic plants showing symptoms of growth inhibition, along with 2 kg of soil adhering to the roots, were collected from a plantation located in the Łódź Voivodeship, Łowicz County, in 2022. After being transported to the laboratory, the plants were separated from the adhering soil and thoroughly cleaned. Nematodes were isolated from the soil using a centrifugation method, while the nematodes from the bulbs and leaves were isolated using a modified Baermann method [van Bezooijen 2006]. The isolated specimens were counted using an OLYMPUS SZ-60 stereomicroscope.

Morphological and molecular identification

For species identification, adult females and males were preserved in TAF and mounted in permanent microscope slides according to the method of Hooper and Evans [1993]. Measurements were made using an Axioskop 2plus biological microscope with an Axiocam MRm camera and ZEN image analysis software. Nematodes were identified at the species level using the Baranovskaya key [1981].

For genetic identification of the species, the females of the studied nematode were placed in a DESS solution for DNA preservation [Yoder et al. 2006]. Subsequently, DNA was isolated from individual specimens using a lysis buffer [Holterman et al. 2006]. The obtained lysate was either immediately used for PCR reactions or stored at –20 °C. In the PCR reaction, primers 988F (5′-ctcaaagattaagccatgc-3′) and 1912R (5′-tttacggtcagaactaggg-3′) were used to amplify a fragment of the 18S rDNA gene (SSU rDNA) [Holterman et al. 2006]. PCR amplifications were sequenced by an ABI 3500L genetic analyzer (Applied Biosystems, Foster City, CA, USA). The obtained sequences were compared with sequences deposited in the National Center of Biotechnology Information (NCBI) using the *Basic Local Alignment Search Tool* (*BLAST*).

RESULTS

Nematodes from the family Aphelenchoididae were isolated from both the soil and the leaves/stems of the garlic plants. From the aboveground parts of the garlic plants, 30 adult and juvenile nematodes were isolated. In the soil, nematodes were found at an average density of 75 individuals per 100 mL. Morphological and morphometric studies revealed that the developing nematode on the garlic plants was *Robustodorus subtenuis.* A comparison of the obtained 18S rDNA sequence with sequences in the NCBI database confirmed the identification of the studied nematodes as *R. subtenuis.* Besides *R. subtenuis,* only two females of *A. rhitzemabosi* were isolated from the leaves/stems of the garlic plants.

Morphometric analysis of individuals isolated from both the soil and plant stems showed that both females and males of *R. subtenuis* differ from previously studied populations originating from ornamental plants (Tabs 1 and 2). Female nematodes have on average shorter bodies and longer tails compared to previously observed individuals, with the averages calculated for the soil nematode population being slightly lower than those for the nematodes from the plant stems. Furthermore, females found in the soil were shorter from the lip region to the vulva and had a shorter vulval sac, with its length accounting for 32% to 45% of the distance from the vulva to the anus. Similar to the females, the bodies of the males are, on average, shorter and have longer tails. The bodies of the males from the garlic sample are wider at the level of the anus. The average body length of females from the soil is shorter than individuals from the plants.

DISCUSSION

Among plant-parasitic nematodes of the so-called ‘leaf nematodes’ group, the most important species negatively affecting the proper growth of garlic is the plant parasitic nematode *D. dipsaci* [Bélair et al. 2018, Bird et al. 2018, Cid del Prado-Vera et al. 2018, Chitambar et al. 2018, Chen and Grabau 2018, MacGuidwin 2018, Pokharel 2018, Westphal et al. 2018, Zasada et al. 2018]. It is a pest with a wide distribution. Its presence in the soil on a plantation can lead to damage reaching up to 100% of crop [Bird et al. 2018]. To date, the presence of *R. subtenuis* in garlic plants has not been reported, although the species has previously been isolated mainly from the bulbs of ornamental plants. From garlic crops suspected of nematode infestation, *A. fragariae* [Pedroche et al. 2013] or *A. varicaudatus* [Kusuma et al. 2020] have also previously been isolated.

There is a lack of data regarding the biology of *R. subtenuis.* It can only be assumed that under favor-

Table 1. Morphometric data of *Robustodorus subtenuis* (Cobb, 1926) Kanzaki et al. 2018 females from garlic (*Allium sativum* L.) plantations

Source	Goodey [1935]	Allen [1952]	Paramonov [1964]	EPPO [2017]	Deimi et al. [2006]	This research	
Plant species	*Narcissus*	–	–	–	*Gladiolus grandiflorus* L.	*Allium sativum* L.	
Sample	Bulbs	–	–	–	Corms and pseudostems	Soil	Leaves
n	–	–	–	–	10	7	21
L	820–1300	870–1150	820–1300	690–1100	822 ±77.4 (694–927)	538 ±48 (532–606)	690 ±56 (603–784)
a	44–66	44–57	39–66	45–68	51.8 ±5.8 (43.4–63.8)	28.5 ±1.9 (26.6–31.9)	29.7 ±2.1 (24.8–33.9)
b	12.5–19.0	12–17	12–19	–	–	–	–
b'	–	–	–	–	6.3 ±0.7 (5.1–7.4)	4.5 ±0.7 (3.5–5.5)	4.8 ±0.4 (4.3–5.4)
c	27.5–32.5	24–28	22–32.5	22–28	34.9 ±4.2 (29.3–41.1)	14.8 ±1.0 (13.5–16.8)	16.9 ±1.7 (14.0–20.1)
c'	–	–	–	–	2.5 ±0.2 (2.2–2.8)	3.6 ±0.4 (2.9–4.1)	3.3 ±0.4 (2.7–4.1)
Stylet length	11–12	11	8–11	11–13	11.6 ±0.5 (11.0–13.0)	11.0 ±0.5 (11.0–12.0)	11.2 ±0.4 (11.0–12.0)
Pharynx length with glands	–	–	–	–	130 ±12.3 (120–157)	122 ±12 (100–136)	144.3 ±12.8 (134–175)
Excretory pore from lip region	–	–	–	–	–	77.1 ±7.1 (65.0–85.0)	81.6 ±6.1 (71–97)
Lip region–vulva length	–	–	–	–	585 ±50.8 (498–645)	371 ±36 (297–430)	487 ±43 (428–570)
Vulva–anus length (v–a)	–	–	–	–	–	130.6 ±12.8 (104–145)	162.6 ±17.3 (113–193)
Maximum body diameter	–	–	–	–	16 ±2 (14.5–20)	18.9 ±1.5 (16.0–21.0)	23.3 ±2.3 (18.0–30.0)
Tail length	–	–	–	–	22.8 ±2.4 (20–27.3)	36.4 ±2.3 (32–40)	40.9 ±3.2 (38–45)
Anal body diameter	–	–	–	–	9.3 ±0.7 (8.5–10)	10.3 ±0.9 (9–12)	12.4 ±1.0 (11.0–15.0)
Postvulval uterine sac (PUS)	–	–	–	–	115 ±19.3 (80–138)	52.6 ±4.1 (45–58)	90.6 ±15.4 (68–118)
PUS – % v–a	About 50%	–	1/3	58–80	68 ±6.6 (58–80)	40.5 ±3 (36.2–45.1)	56.2 ±11.1 (48–88.5)
PUS – xV	–	–	–	–	–	7.1 ±0.5 (6.5–7.8)	5.5 ±0.8 (3.7–6.9)
V%	65.9–73.5	–	65.9–78	–	71.3 ±0.9 (69.6–72.6)	68.9 ±1.3 (67.4–71.0)	70.5 ±1.7 (66.2–75.7)

Table 2. The morphometric data of males of *Robustodorus subtenuis* (Cobb, 1926) Kanzaki et al. 2018 from garlic (*Allium sativum* L.) plantations

Source	Goodey [1935]	Allen [1952]	Paramonov [1964]	EPPO [2017]	Deimi et al. [2006]	This research	
Plant species	*Narcissus*	–	–	–	*Gladiolus grandiflorus* L.	*Allium sativum* L.	
Sample	bulbs	–	–	–	corms and pseudostems	soil	leaves
n	–	–	–	–	19	10	11
L	750–1010	870–950	750–1010	–	841 ±74.4 (745–975)	542 ±38 (488–608)	600 ±40 (530–672)
a	54.5–66	57–68	54.5–66	–	56.3 ±3.4 (50–60.2)	30.3 ±1.8 (27.7–33.9)	31.3 ±2.4 (27.2–34.1)
b	11.0–17.8	12–14	11–17.8	–	–	–	–
b'	–	–	–	–	6.3 ±0.8 (5.3–7.9)	3.8 ±0.4 (3.2–4.3)	4.4 ±0.4 (3.9–5.4)
c	23–26	21–28	23–26	–	34.9 ±4.2 (29.3–41.1)	15.8 ±1.2 (14.0–18.4)	16.9 ±1.1 (15.1–18.7)
c'	–	–	–	–	2.5 ±0.2 (2.2–2.9)	2.7 ±0.2 (2.2–2.9)	2.9 ±0.2 (2.5–3.3)
Stylet length	11–12	11	8–11	–	11.1 ±1.1 (9.0–13.0)	11.1 ±0.2 (11.0–11.5)	11.0 ±0.4 (10.1–12.0)
Pharynx length with glands	–	–	–	–	134 ±9.3 (123–149)	146 ±16 (120–170)	136 ±10 (120–150)
Excretory pore from lip region	–	–	–	–	–	74 ±7 (64–85)	77 ±6 (65–86)
Lip region–anus length	–	–	–	–	–	508 ±37 (455–575)	564 ±39 (495–610)
Maximum body diameter	–	–	–	–	15 ±1.8 (13.0–18.0)	17.9 ±0.9 (16.0–19.0)	19.4 ±1.4 (17–21)
Tail length	–	–	–	–	24.2 ±1.4 (21.8–26.4)	34.5 ±2.2 (32–38)	35.4 ±1.9 (33–39)
Anal body diameter	–	–	–	–	9.8 ±0.5 (9.0–11.0)	13.0 ±1.2 (12–15)	12.4 ±1.0 (11–14)
Spicule length (as curved median line)	–	–	–	20–21	20.2 ±1.4 (18.2–22.7)	19.7 ±1.0 (18–21)	19.7 ±1.0 (18–21)
Spicule length (as straight line from apex to tip of dorsal limb)	–	–	–	–	16.7 ±1.1 (15–18)	20.0 ±1.5 (17.0–22.0)	17.0 ±0.5 (16.0–17.5)

able conditions of temperature and humidity, symptoms of infestation similar to those caused by the feeding of *D. dipsaci* will appear on the plant stems. Since there are known cases of asymptomatic root infestation by *R. subtenuis* with no visible changes to the aboveground parts of narcissus plants [Mor and Spiegel 1993], it can be inferred that this nematode may feed on garlic roots without altering the appearance of the plant stems. The obtained results indicate the need to monitor garlic crops for the presence of this nematode species as well.

Morphometric observations between populations of *R. subtenuis* previously isolated from *Narcissus* and *Gladiolus grandiflorus* L. revealed intraspecific and interpopulation morphometric variability of this nematode. This can be explained by the interaction between the plants and the nematodes of this species feeding on them. Thorne [1961] and Dobosz et al. [2020] for *D. destructor*, as well as Kaur and Attri [2013] for *M. incognita*, have previously suggested that the variation in morphometric and anatomical traits of plant-parasitic nematodes can be influenced by their host plants.

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