Acta Sci. Pol. Hortorum Cultus, 21(5) 2022, 153–161

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2022.5.13

ORIGINAL PAPER

Accepted: 14.07.2022

FIRST REPORT OF THE *Rotylenchus agnetis* SZCZYGIEŁ, 1968, *Rotylenchus pumilus* PERRY, 1959 AND *Paratylenchus nanus* COBB, 1923 ASSOCIATED WITH *Miscanthus × giganteus* J. M. GREEF & DEUTER EX HODK. & RENVOIZE IN UKRAINE

Andrzej Skwiercz[1], Tatyana Stefanovska[2], Miloslav Zouhar[3], Valentina Pidlisnyuk[4], Łukasz Flis[5]✉

[1] The National Institute of Horticultural Research, ul. Konstytucji 3 Maja 1/3, 96–100 Skierniewice, Poland

[2] Department of Entomology, Integrated Pest Management and Plant Quarantine, The National University of Life and Environmental Sciences, Heroiv Oborony St, 15, 03041 Kyiv, Ukraine

[3] Department of Plant Protection, Czech University of Life Sciences, Kamýcká 129, 165 00 Praha-Suchdol, Czech Republic

[4] Department of Environmental Chemistry and Technology, Jan Evangelista Purkyne University in Usti nad Labem, Pasteurova 3544/1, 400 96 Ústí nad Labem-město, Czech Republic

[5] Museum and Institute of Zoology, Polish Academy of Sciences, Wilcza 64, 00-679 Warsaw, Poland

ABSTRACT

During a survey of plant parasitic nematodes associated with *Miscanthus* × *giganteus* which was carried out in Ukraine in 2016–2017, an occurrence of *Rotylenchus agnetis* Szczygieł, 1968, *Rotylenchus pumilus* Perry, 1959 and *Paratylenchus nanus* Cobb, 1923 was found. To the best of our knowledge this is the first record of a parasite species which can damage *M.* × *giganteus*. The species were described morphologically and using molecular tools. Further profound study on pathogenicity of those species is needed.

Key words: plant-feeding nematodes, *Miscanthus* × *giganteus*, morphological description, molecular identification

INTRODUCTION

Miscanthus × *giganteus* J.M. Greef & Deuter ex Hodk. & Renvoize, (Poacea = Gramineae) is a rhizomatous, perennial C4 grass species which originated in South-East Asia [Lewandowski et al. 2018]. The sterile clone *M.* × *giganteus* is a high-yielding genotype and currently the standard cultivar in commercial utilization in Ukraine [Kvak et al. 2018]. It has an adequate environmental profile with a highly adaptive potential, namely to grow in marginal soils as well as those of different anthropogenic origins, slightly contaminated by trace elements [Nebeská et al. 2019, Pidlisnyuk et al. 2020]. *M.* × *giganteus* is among the most promising sources of biomass for direct energy production and bio-based products [Fradj et al. 2020]. There is a significant acreage of available marginal and abandoned lands in Ukraine where traditional agriculture is not profitable [Triboy and Dragnev 2018]. Simultaneously, the growing demands for obtaining lignocellulose biomass are good drivers for upscaling commercial production of *M.* × *giganteus* in the country. Since the first *M.* × *giganteus* plantation was established in Ukraine in 2006, the production has rapidly expanded and currently, its crop area covers up to 1,500 ha [Roik et al. 2019]. As miscanthus plots switch from

✉ lflis@miiz.waw.pl

small fields to large commercial plantations in different agroecological zones, nematodes, plant pathogens and pests are beginning to emerge [Mekete et al. 2011, Stefanovska et al. 2021].

Among plant parasitic nematodes several species, including spiral *Rotylenchus* Steiner, 1945 and pin *Paratylenchus* [Micoletzky 1922] were shown as species associated with Poaceae family [Háněl 2017, Ghaderi 2019]. If the area of *M.* × *giganteus* that also belongs to Poaceae family increases, these nematode might parasitise the crop and cause the yield reduction. Obligate plant parasitic spiral nematodes of the genus *Rotylenchus* (Nematoda: Haplolaimidae) are represented by 103 valid species widely distributed across the world. In terms of *Rotylenchus* species numbers, Europe ranks second to Asia [Castillo and Vovlas 2005]. Several species are considered economically significant for agriculture like *R. robustus* [De Man 1876], *R. buxophilus* [Golden 1956], *R. uniformis* [Thorne 1949, Loof and Oostenbrink 1958], and *R. goodeyi* (Loof and Oostenbrink, 1958) [Nguyen et al. 2019]. *Rotylenchus agnetis* Szczygieł, 1968 is known as semi-endoparasitic nematode. The species is able to enter roots using longer stylet compared to other migratory parasites of the same genus which commonly grow on roots causing necrosis [Decraemer and Hunt 2006]. According to the Nemataxa database*,* *R. agnetis* is monophagous and known to have parasitised butcher's broom *Ruscus aculeatus* L. (Asparagaceae) only. However, several studies detected this nematode species also in the rhizosphere of forest plants [Skwiercz 2012], fruit trees [Lišková et al. 2007], grapevine *Vitis vinifera* L. (Vitaceae) [Skwiercz et al. 2015], barley *Hordeum vulgare* L. (Poaceae), different ornamental nursery plants and woods [Winiszewska et al. 2012].

At first female specimens of *R. agnetis* were found in strawberry *Fragaria ananassa* L. (Rosaceae) plantations in Poland and described by Szczygieł [1968]. The next description of isolated *R. agnetis* from apple *Malus domestica* L. Borkh (Rosaceae) was elaborated in Moldova by Nesterov [1979]. Baydulova [1981] added information on morphology of this species based on specimens isolated in Kazakhstan from *Medicago sativa* L. (Fabaceae). The description of *R. agnetis* in forest nurseries was done by Peneva and Nedelchev [1992]. These authors made the significant contribution to the clarification of taxonomic position of the species by presenting the morphological and morphometric description of *R. agnetis* isolated from different hosts and locations in Bulgaria. Geraert and Barooti [1996] decribed *R. agnetis* recorderd for the first time at a pine tree *Pinus sylvestris* L. (Pinaceae) plantation in Iran and gave additional data based on a scanning electron microscope study. The latest description of *R. agnetis* isolated from the rhizosphere of butcher's broom was provided by Vovlas et al. [1998] in Italy.

The available data on *Rotylenchus pumilus* Perry, 1959 host range related to forest plants is very limited. Yuen [1966] found a large population of *R. pumilus* co-occurring with *Helicotylenchus vulgaris* Yuen, 1964 in soil under grasses in meadows adjacent to forests. Riffle [1972] detected *R. pumilus* in soil under pine trees and junipers *Juniperus communis* L. (Cupressaceae) in South Mexico. Furthermore, in a pot experiment he found that although that nematode species parasitised the pine tree it did not lead to a significant reduction in seedling growth. Germani and La Massese [2002] also described *R. pumilus* which was collected in the rhizosphere of pine trees in France. Skwiercz [2012] detected the species as inhabiting the nursery of forest plants and colonizing the jerusalem artichoke *Helianthus tuberosus* L. (Asteraceae) [Zapałowska and Skwiercz 2018] in Poland. There are several records on *R. pumilus* that were observed in grapevines: by Kankina and Teben'kova [1980] in Tajikistan; Katalan-Gateva [1980] in Bulgaria and Skwiercz et al. [2015] in Poland.

The existence of a significant number of *Rotylenchus* species complicated the process of its pragmatic identification due to the creation of tabular and dichotomous keys following morphological features [Cantalapiedra-Navarrete et al. 2013].

Pin nematodes of the genus *Paratylenchus* are obligate ectoparasites. In Europe they are represented by 32 species [Castillo and Vovlas 2007]. *Paratylenchus nanus* Cobb, 1923 is closely related to *Paratylenhcus bukowinensis* Micoletzki, 1922 from which it differs according to Brzeski [1998]. *P. nanus* has got pronounced annulation on dorsal side near terminus tail while *P. bukowinensis* has got indistinct annuli often small near tail end. Males *P. nanus* without stylet vs. males *P. bukowinensis* with weak stylet [Brzeski

1998]. The species of these genera are widely distributed in crops and natural vegetation worldwide [Stefanovska 1992]. *P. nanus* is known to be associated with perennial grasses cultivated in meadow soils [Brzeski 1998], golf cources in the rhizosphere of cereals and orchards [Viketoft et al. 2005]. The species is distributed widely in perennial crops, all above grass stands, hop gardens, orchards or forest trees and shelterbelts [Wasilewska 2006, Čermák and Renčo 2010, Kozlovskyi 2012]. Italian ryegrass *Lolium multiflorum* L., perennial ryegrass *Lolium perenne* L. and cocksfoot *Dactylis glomerata* L. are suitable hosts for *P. nanus* [Watson and Bell 2001, Ciobanu et al. 2003]. Kozlovskyi [2012] recorded *P. nanus* in deciduous and mixed forests located in the Carpathians, showing that the species is not restricted to lowland habitats and grass vegetation. Several descriptions of *P. nanus* are available, however, there are some controversies about it. Morphological identification of pin nematodes is rather difficult due to their smaller size, problems associated with separation of very similar diagnostic characteristics, and high variability under influence of environmental factors [Brzeski and Hanel 2000]. Ghaderi et al. [2016] listed important morphological characteristics which facilitate the process of morphological identification accuracy, i.e. number of lateral lines, length of the stylet and the presence/absence of vulval flap. In order to avoid the complication with morphometric indentification of *Rotylenchus* spp. and *Paratylenchus* spp., the alternative DNA based approach has been suggested recently [Cantalapiedra-Navarrete et al. 2013, Van den Berg et al. 2014].

During a nematological survey conducted at six locations and four soil types across Ukraine in 2016–2017, soil samples under *M. × giganteus* grown in 1–10 age plantations were collected. The study of nematode specimens extracted from these samples revealed the presence of 53 plant-feeding nematode species. Among frequently occurring in this energy crop species, *R. agnetis*, *R. pumilus*, *P. nanus* were recorded. Because these nematodes were recorded for the first time in *M. × giganteus* plantations in Ukraine, characterisation of populations from Ukraine was needed. The objective of this study was to combine the morphological and molecular approaches in order to identify *R. agnetis*, *R. pumilus*, and *P. nanus*. Connecting the morphological and molecular species concepts might facilitate species identification within the genus *Rotylenchus*, *Paratylenchus*.

MATERIAL AND METHODS

Nematodes were isolated from soil samples collected to a depth of about 20–40 cm and taken randomly in autumn from different plots of *M. × giganteus*. Plants did not have symptoms of nematode infection. The map with locations where *R. agnetis*, *R. pumilus* and *P. nanus* were detected is featured in Figure 1. Samples were collected at locations described in each species data list (Tab. 1). Nematodes were isolated using the modified Baermann and centrifugal flotation techniques [Hooper et al. 1962]. Extracted nematodes for the further morphological study were heat-killed, fixed in TAF and processed to glycerol. Identification and measurements of specimens were made on prepared temporary slides by using light microscopy. For the identification and comparison with original descriptions of the nematode species detected in this study, several diagnostic keys were used, i.e.: Perry et al. [1959], Sher [1961], Hooper [1962], Geraert [1965], Decraemer [1995], Brzeski [1998] and Andrássy [2007].

Extraction of DNA from fixed nematodes was done by Exgene Genomic DNA micro kit. The specimens were removed from the fixative solution and were individually kept in 10 µm of extraction buffer and processed in accordance with the manufacturer's instructions. Total DNA from single nematodes was amplified by the protocol described by Douda et al. [2013], using two prepared fragments, i.e.: the first one using the primers D2A (5´-ACAAGTACCGTGAGGGAAAGTTG-3´) and D3B (5´-TCGGAAGGAACCAGCTACTA-3´) [Ellis et al. 1986, Courtright et al. 2000] for the 28S large sub-unit rRNA, S18 (5′-TTGATTAGGTCCCTGCCCTTT-3′) and S28 (5′-TTTCACTCGCCGTTACTAAGG-3′) situated inside the cistron rDNA including region ITS1-5.8S-ITS2 rDNA (S18 S28) [Ellis et al. 1986]. The PCR products were separated by 2% agarose gel electrophoresis and purified by GeneJET Gel Extraction Kit (Thermo Fisher Sci.). Three PCR products were prepared from every single nematode and sequenced. The DNA sequencing was performed in GATC Biotech (Germany). Obtained sequences were processed by free avail-

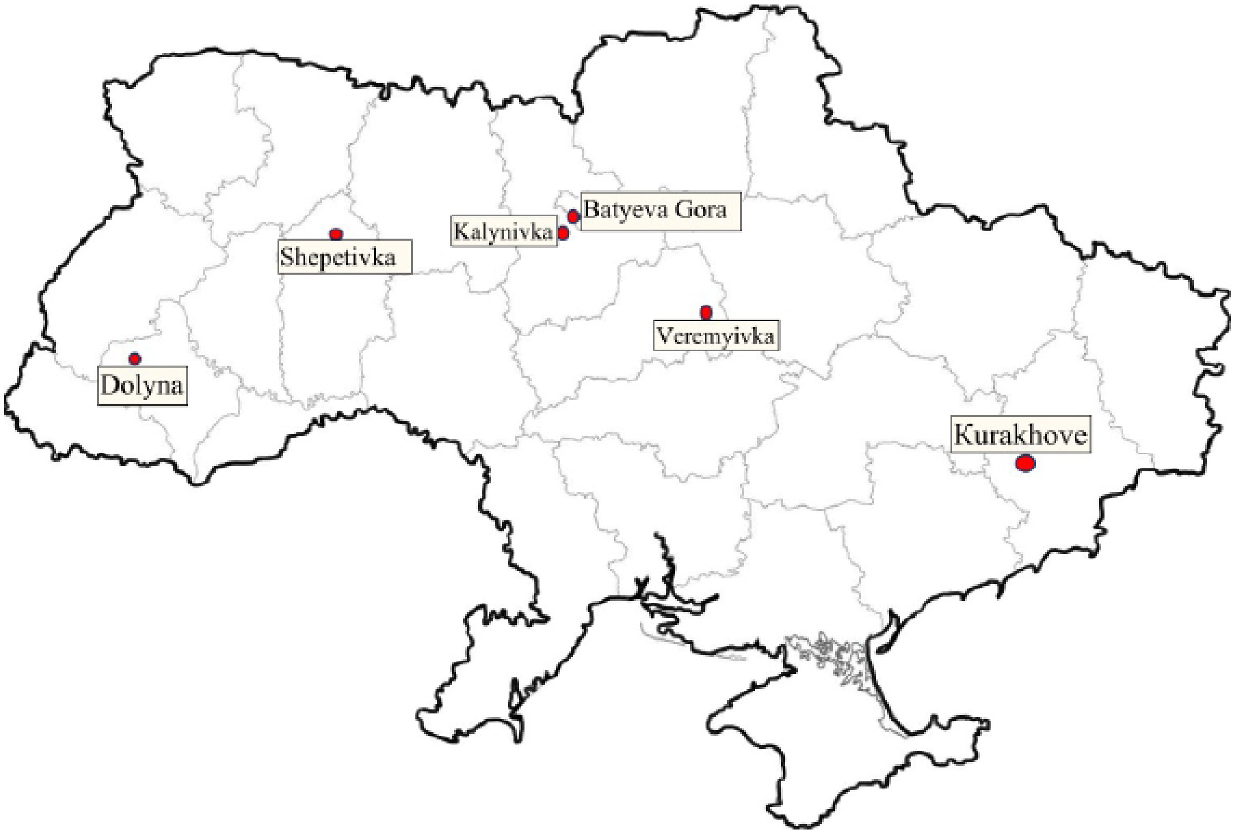

Fig. 1. The map of Ukraine with locations where *Rotylenchus agnetis* Szczygieł, 1968*, Rotylenchus pumilus* Perry, 1959 and *Paratylenchus nanus* Cobb, 1923 were detected

able software BioEdit and consensual sequences were assembled. The BLAST algorithm for screening of obtained data was used. [Sayers et al. 2011].

RESULTS AND DISCUSSION

Rotylenchus agnetis Szczygieł, 1968

Female, n: 12. L = 800 (640–960) µm, c = (46–64); c' = 0,7 (0,5–0,9) µm stylet = 32 (28–34) µm, pharynx = 112 (106–118) µm. V = 59 (56–62); tail = 13 (10–16) µm; tail annuli = 7 (6–8), phasmids at 4 annuli anterior to 2 annuli posterior anus. Body is loosely spiral, head offset by constriction with 5–7 annuli. Excretory pore is opposite to the pharynx and intestine junction. Pharyngeal lobe 0,8–1,0 µm body width long. In the female reproductive system spermatheca is filled with sperm. Phasmids are 4 annuli anterior to 2 annuli posterior anus. Tail hemispherical bearing 6–8 annuli, on the tip two widely annuli.

The female length was significantly less than recorded by Geraert and Barooti [1996] but it was almost in agreement with Szczygieł [1968], Nesterov [1979] and Peneva and Nedelchev [1992]. The stylet was longer in comparison with those reported by Szczygieł [1968], Geraert and Barooti [1996], Peneva and Nedelchev [1992], however fitted with measurements of *R. aceri* − a species synonymized with *R. agnetis* was done by Berezina [1985]. The tail length was in the correspondence with Peneva and Nedelchev [1992] and Geraert and Barooti [1996]. A compilation of the numbers of specimens of this species detected in the samples is presented in Table 1.

Rotylenchus pumilus Perry, 1959

Female, n: 8. L = 622 (495–750) µm, c' = 0,9 (0,9–1,0); stylet: 32 (24–28) µm, pharynx: 102,5 (95–110) µm, V = 59 (58–60); tail = 14 (12–16) µm. Body is small, tightly spiral, location of the lateral field is irregular. Head bearing 4–5 annuli, not offset, with strongly refractive constriction. Pharyngeal lobe overlapped intestine for 0,6–1,0 µm body width. Spermatheca is filled with sperm. Tail rounded, convex on the dorsal side, equal of anal body diameter.

The three morphological features of *R. pumilus* are not in agreement with measurements of those species detected in the rhizosphere under pine trees [Germani and La Massese 2002], namely: female length is smaller; the stylet and tail are slightly larger. However, if compared to the description of *R. pumilus* by Perry et al. [1959] and Sher [1961], in our records the body length and stylet are shorter, but the tail is longer.

A compilations of the numbers of specimens of this species detected in the samples is presented in Table 1.

Paratylenchus nanus Cobb, 1923

Female, n: 15. L = 370 (320–420) µm, stylet: = 26 (24–28) µm, pharynx = 94 (90–98) µm, tail = 22 (18–26) µm, c = 15 (12–18), c'= 2,7 (2,0–3,4); V = 83 (82–84). Female bent ventrad, head rounded, excretory pore is 21–25% of body length. Spermatheca with sperm, vulval claps rounded, distinct.

Morphological features values in our description of *P. nanus* fitted with original description presented

Table 1. Habitat and number of investigated species from the plantation of *Miscanthus × giganteus* J.M. Greef & Deuter ex Hodk. & Renvoize

Species	Locality	Age of plantation (years)	Soil type	Number of samples	Number of samples with the investigated species	% of samples with the investigated species	Number of specimens of plant parasitic nematodes	Number of specimens of investigated species	Number of specimens of investigated species/unit of soil
Rotylenchus agnetis	Szepetivka 50°07'53.5"N 27°13'58.3"E	2	Chernozem	4	1	25	558	90	90/100 g
	Batyeva Gora 50°25'04.9"N 30°29'55.0"E	7	Clayic podzoluvisoil	4	1	25	1080	140	140/100 g
	Veremyivka 49°36'51.9"N 33°15'09.8"E	5	Chernozem	4	1	25	738	25	25/100 g
	Kalynivka 50°13'45.8"N 30°13'09.0"E	8	Chernozem	4	1	25	271	90	90/100 g
	Kurakhove 47°58'30.5"N 37°19'28.7"E	1	Haplic chernozem	4	1	25	465	120	120/100 g
	Dolyna 48°58'14.8"N 23°58'59.9"E	1	Clayic cambisoils	4	1	25	715	150	150/100 g
Rotylenchus pumilis	Szepetivka	2	Clayic cambisoils	4	1	25	558	150	150/100 g
	Batyeva Gora	5	Clayic podzoluvisoil	4	1	25	738	96	96/100 g
	Batyeva Gora	7	Clayic podzoluvisoil	4	2	50	1080	206	206/100 g
Paratylenchus nanus	Szepetivka	2	Chernozem	4	2	50	558	300	300/100 g
	Szepetivka	3	Chernozem	4	2	50	1074	300	300/100 g

by Thorne and Smolik [1971] and Nouri et al. [2004] with the exception of body length. It is shorter in comparison with maesurements made by Torn and Smolik [1971].

A compilation of the numbers of specimens of this species detected in the samples is presented in Table 1.

Molecular characterization

It should be noted that not all cases obtained sufficient quality DNA to amplify all the desired regions, which may be due to nematode fixation and also a small amount of biological material because the DNA was isolated from a single nematode at a time.

In the case of *R. pumilus* and *P. nanus*, the obtained sequences of the small and large ribosomal DNA subunit sections were compared using the BLAST algorithm with the NCBI database and the sequences showed more than 99% similarity with the sequences listed in the database. The species affiliation determined using morphological data was confirmed by analysis of the nucleotide sequences obtained. In the case of *R. agnesis*, it was not possible to confirm the morphological data by molecular biological sequence analysis because the only DNA segment available for comparison in the NCBI database, the 28S ribosomal RNA gene, could not be amplified.

CONCLUSION

Three nematode species: R. agnetis, R. pumilus and P. nanus have been recorded for the first time in *M. × giganteus* plantations located in two agroecological zones: Forestry Steppe and Steppe in Ukraine and characterized by four types of soil. Those three species were characterized morphometrically and two species using molecular methods. The description prepared of *R. agnetis* specimens detected on *M. × giganteus* (perennial grass of Poacea family) extends the body of morphological data as previously it covered the forest plants only. The detection of *P. nanus* on this perennial grass confirmed a preference displayed by this species for inhabiting the rhizosphere of grasses. There is a likelihood that this species could have been distributed while planting *M. × giganteus* stands as the acreage was increaing. The morphological and molecular descriptions of the three plant-feeding nematode species will facilitate the overcoming of the current nematode threats through their identification and contribute to the spiral and pin nematodes' biodiversity information.

SOURCE OF FUNDING

This research was partly supported by NATO SPS G4687.

REFERENCES

Andrássy, I. (2007). Free-living nematodes of Hungary (*Nematoda errantia*). Vol. 2. In: Pedozoologia Hungarica No. 4, Csuzdi, Cs., Mahunka, S. (eds.). Hungarian Natural History Museum and Systematic Zoology Research Group of HAS, Budapest, 496 pp.

Baydulova, L.A. (1981). Nematodes of the family Hoplolaimidae in Western Kazakhstan. Parasitologiya, 15, 83–86 [in Russian].

Berezina, N.B. (1985). A new species *Rolylenchus aceri* n. sp. (Nematoda: Tylenchida) from the rhizosphere of *Acer campestre* (L.). Bull. Vsesoy. Inn Gel'mint. K.I. Skryabina, 41, 89–91 [in Russian].

Brzeski, M.W., Hanel, L. (2000). Paratylenchinae: evaluation of diagnostic morpho-biometrical characters of females in the genus *Paratylenchus* Micoletzcy, 1922 (Nematoda: Tylenchulidae). Nematology, 2, 253–261.

Brzeski, M.W. (1998). Nematodes of Tylenchina in Poland and temperate Europe. Wyd. Museum i Instytut Zoologii PAN, Warszawa, Poland, 396 pp.

Cantalapiedra-Navarrete, C., Navas-Cortés, J.A., Liébanas G., Vovlas N., Subbotin S.A., Palomares-Rius, J.E., Castillo, P. (2013). Comparative molecular and morphological characterisations in the nematode genus *Rotylenchus*: *Rotylenchus paravitis* n. sp., an example of cryptic speciation. Zool. Anz. J. Comp. Zool., 252(2), 246–268. https://10.1016/j.jcz.2012.08.002

Castillo, P., Vovlas, N. (2005). Bionomics and identification of the genus *Rotylenchus* (Nematoda: Hoplolaimidae). Leiden, Brill Academic Publishers, pp. 377.

Castillo, P., Vovlas, N. (2007). *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis, biology, pathogenicity and management. Nematology Monographs and Perspectives. Brill, Leiden–Boston, 529 pp.

Čermák, V., Renčo, M. (2010). The family Paratylenchidae Thorne, 1949 in the rhizosphere of grass and woody species in Europe: a review of the literature. Helminthologia, 47(3), 139–146. https://10.2478/s11687-010-0021-z

Ciobanu, M., Geraert, E., Popovici, I. (2003). The genera *Paratylenchus* Micoletsky, 1922 and *Gracilacus* Raski, 1962 in Romania (Nematoda: Tylenchulidae). Nematol. Mediterr., 31, 55–59.

Cobb, N.A. (1923). Notes on *Paratylenchus*, a genus of nemas. J. Wash. Acad. Sci, 13, 251–7.

Courtright, E.M., Wall, D.H., Virginia, R.A., Frisse, L.M., Vida, J.T., Thomas, W.K. (2000). Nuclear and mitochondrial DNA sequence diversity in the antarctic nematode *Scottnema lindsayae*. J. Nematol., 32(2), 143–153.

De Man, J.G. (1876). Onderzoekingen over vrij in de aarde levende Nematoden. Tijdschrift der Nederlandsche Dierkundige Vereeniging Ver 2, 78–196.

Decraemer, W. (1995). The family Trichodoridae: stubby root and virus vector nematodes. Kluwer Academic Publishers, 360 pp.

Decraemer, W., Hunt, D.J. (2006). Structure and classification. In: Plant nematology, Perry, R.N., Moens, M. (eds.). CABI, Oxfordshire, pp. 3–32. https://doi.org/10.1079/9781845930561.0003

Douda, O., Marek, M., Zouhar, M., Ryšánek, P. (2013). Insights into the structure and phylogeny of the 28S rRNA expansion segments D2 and D3 of the plant-infecting nematodes from the genus *Ditylenchus* (Nematoda: Anguinidae). Phytopathol. Mediterr., 52(1), 84–97. https://doi.org/10.14601/Phytopathol_Mediterr-11334

Ellis, R.E., Sulston, J.E., Coulson, A.R. (1986). The rDNA of *C. elegans*: sequence and structure. Nucl. Acids Res., 14, 2345–2364.

Fradj, N.B., Rozakis, S., Borzęcka, M., Matyka, M. (2020). Miscanthus in the European bio-economy: a network analysis. Ind. Crops Prod., 148, 112281. https://doi.org/10.1016/j.indcrop.2020.112281

Geraert, E. (1965). The genus *Paratylenchus*. Nematologica, 11(3), 301–334. https://doi.org/10.1163/187529265X00221

Geraert, E., Barooti, S. (1996). Four *Rotylenchus* from Iran, with a key to the species. Nematologica, 42(5), 503–520. https://doi.org/10.1163/004625996X00018

Germani, G., La Massese, C.S. (2002). Description of four new species and two populations of *Rotylenchus* (Nematoda: Hoplolaimidae). Nematol. Mediter., 30(2), 203–208.

Ghaderi, R. (2019). The damage potential of pin nematodes, *Paratylenchus* Micoletzky, 1922 sensu lato spp. (Nematoda: Tylenchulidae). J. Crop Prot., 8(3), 243–257.

Ghaderi, R., Geraert, E., Karegar, A. (2016). The Tylenchulidae of the world; identification of the family Tylenchulidae (Nematoda: Tylenchida). Academia Press, Ghent, Belgium, 453 pp.

Golden, A.M., 1956. Taxonomy of the spiral nematodes (*Rotylenchus* and *Helicotylenchus*), and the developmental stages and host-parasite relationships of *R. buxophilus*, n. sp., attacking boxwood. Bulletin A-85, University of Maryland, MD, USA, Agricultural Experimental Station, 1–28.

Háněl, L. (2017). Soil nematodes in alpine meadows of the Tatra National Park (Slovak Republic). Helminthologia, 54(1), 48–67. https://doi.org/10.1515/helm-2017-0005

Hooper, D.J. (1962). Three new species of *Trichodorus* (Nematod: Dorylaimoidea) and observation on *T. minor* Colbran, 1956. Nematologica, 7, 273–280.

Kankina, V.K., Teben'kova, T.M. (1980). Nematodes from the family Hoplolaimidae (Filipjev, 1934) Wieser, 1953 on grapevine in Tadzhikistan. Izvestiya Akademii Nauk Tadzhikskoi SSR (Ahboroti Akademijai Fanhoi RSS Tocikiston), Biologicheskie Nauki, 1, 33–40 [in Russian].

Katalan-Gateva, S. (1980). Ectoparasitic nematodes of the family Hoplolaimidae Filipjev, 1934 found in the rhizosphere of the vine (*Vitis vinifera* L.). Acta Zool. Bulg., 14, 59–63.

Kozlovskyi, M.P. (2012). Antropogenous transformation of soil invertebrate communities in secondary ecosystems of the Ukrainian Carpathians. Stud. Biol., 6, 135–142. https://doi.org/10.30970/sbi.0601.179

Kvak, V., Stefanovska, T., Pidlisnyuk, V., Alasmary, Z., Kharytonov, M., (2018). The long-term assessment of *Miscanthus × giganteus* cultivation in the forest-steppe zone of Ukraine. INMATEH-Agric Eng., 54(1), 113–121.

Lewandowski, J., Clifton-Brown, A., Kiesel, A., Hastings, Y., Iqbal, Y. (2018). *Miscanthus*. In: Perennial grasses for bioenergy and bioproducts. Production, uses, sustainability and markets for giant reed, miscanthus, switchgrass, reed canary grass and bamboo, Alexopoulou, E. (ed.). Academic Press, London, 35–39. 10.1016/B978-0-12-812900-5.00002-3

Lišková, M., Sasanelli, N., D'Addabbo, T. (2007). Some notes on the occurrence of plant parasitic nematodes on fruit trees in Slovakia. Plant Prot. Sci., 43(1), 26–32. https://doi.org/10.17221/2349-PPS

Loof, P.A.A.,Oostenbrink, M. (1958). Die identität von *Tylenehus robustus* De Man. Ibidem 3, 34–43.

Mekete, T., Reynolds, K., Lopez-Nicora, H.D., Gray, M.E., Niblack, T.L. (2011). Plant-parasitic nematodes are potential pathogens of *Miscanthus×giganteus* and *Panicum virgatum* used for biofuels. Plant Dis., 95(4), 95413–95418. https://doi.org/10.1094/PDIS-05-10-0335

Micoletzky, H. (1922). Die freilebenden nematoden mit besonderer Berücksichtigung der Steiermark und der Bukowina, zugleich mit einer revision sämtlicher nicht mariner, freilebender nematoden in form yon genus-Beschreibungen und Bestimmungsschlüsseln. Arch. Nat., Berl., 87, 321–650.

Nebeská, D., Pidlisnyuk, V., Stefanovska, T., Trögl, J., Shapoval, P., Popelka, J., Černý, J., Medkow, A., Kvak, V., Malinská, H. (2019). Impact of plant growth regulators and soil properties on *Miscanthus × giganteus* biomass parameters and uptake of metals in military soils. Rev. Environ. Health., 34, 283–291. https://doi.org/10.1515/reveh-2018-0088

Nesterov, P. (1979). Plant-parasitic and free-living nematodes in South-Eastern USSR. Shtiintza, Kishinev, 314 pp.

Nguyen, H.T., Trinh, Q.P., Couvreur, M., Singh, P.R., Decraemer, W., Bert, W. (2019). Description of *Rotylenchus rhomboides* n. sp. and a Belgian population of *Rotylenchus buxophilus* (Tylenchomorpha: Hoplolaimidae). J. Nematol., 5, 1–20. https://doi.org/10.21307/jofnem-2019-023

Nouri, S., Kheiri, A., Karegar, A. (2004). Identification of plant parasitic nematodes fauna (Tylenchida) of fruit trees in Zanjan region. In: Proc. 16th Iran. Plant Prot. Cong., Plant Diseases, Tabriz, Iran, 434.

Peneva, V., Nedelchev, S. (1992). On the morphology and distribution of *Rotylenchus agnetis* Szczygieł, 1968 (Nemata: Hoplolaimidae) in Bulgaria. Fund. Appl. Nematol., 15, 91–96.

Perry, V.G., Darling, H.M., Thome, G. (1959). Anatomy, taxonomy and control of certain spiral nematodes attacking blue grass in Wisconsin. Bull. Wisconsin Agric. Exp. Stat., 207, 1–24.

Pidlisnyuk, V., Shapoval, P., Zgorelec, Ž., Stefanovska, T., Zhukov, O. (2020). Multiyear phytoremediation and dynamic of foliar metal(loid)s concentration during application of *Miscanthus × giganteus* Greef et Deu to polluted soil from Bakar, Croatia. Environ. Sci. Pollut. Res., 27, 31446–31457. https://doi.org/10.1007/s11356-020-09344-5

Riffle, J.W. (1972). Effect of certain nematodes on the growth of *Pinusedulis* and *Juniperus monosperma* seedlings. J. Nematol., 4, 91–94.

Roik, M., Sinchenko, V., Purkin, V., Kvak, V., Gumentik, M. (2019). Miscanthus in Ukraine, Kyiv, Ukraine. FOP Yamchinskiy Press. 256 pp. [in Ukrainian].

Sayers, E.W., Barrett, T., Benson, D.A., Bolton, E., Bryant, S.H., Canese, K., Chetvernin, V., Church, D.M., Di Cuccio, M., Federhen, S., Feolo, M., Fingerman, I.M., Geer, L.Y., Helmberg, W., Kapustin, Y., Landsman, D., Lipman, D.J., Lu, Z., Madden, T.L., Madej, T., Maglott, D.R., Marchler-Bauer, A., Miller, V., Mizrachi, I., Ostell, J., Panchenko, A., Phan, L., Pruitt, K.D., Schuler, G.D., Sequeira, E., Sherry, S.T., Shumway, M., Sirotkin, K., Slotta, D., Souvorov, A., Starchenko, G., Tatusova, T.A., Wagner, L., Wang, Y., Wilbur, W.J., Yaschenko, E., Ye, J. (2011). Database resources of the national center for biotechnology information. Nucl. Acids Res., 39, 38–51.

Sher, S.A. (1961). Revision of the Hoplolaiminae (Nematoda). 1. Classification of nominal genera and nominal species. Nematologica, 6, 155–169.

Skwiercz, A.T. (2012). Nematodes (Nematoda) in Polish forests. I. Species inhabiting soils of nurseries. J. Plant Prot. Res., 52, 169–179.

Skwiercz, A.T., Dzięgielewska, M., Szelągowska, P. (2015). Nematodes in the vineyards in the northwestern part of Poland. Acta Sci. Pol. Hortorum Cultus, 14, 3–12.

Stefanovska, T. (1992). Biological justification of chusing sugar beet parasitic nematodes management practices in the intensive technology of crop production (doctoral dissertation), 161 pp. Retrieved from: http://library.nubip.edu.ua [in Ukrainian].

Stefanovska, T., Skwiercz, A., Zouhar, M., Pidlisnyuk, V., Zhukov, O. (2021). Plant-feeding nematodes associated with *Miscanthus × giganteus* and their use as potential indicators of the plantations' state. Int. J. Environ. Sci. Technol., 1–16. https://doi.org/10.1007/s13762-020-02865-z

Steiner, G. (1945). *Helicotylenchus*, a new genus of plant-parasitic nematodes and its 20 relationship to *Rotylenchus* Filipjev. Proc. Helminth. Soc. Wash., 12, 34–38.

Szczygieł, A. (1968). *Rotylenchus agnetis* sp. n. (Nematoda, Hoplolaimidae) from Poland. Bull. Acad. Pol. Sci. Cl. II ser. Sci. Biol., 16, 573–575.

Thorne, G. (1949). On the classification of the Tylenchida, new order (Nematoda, Phasmidia). Proc. Helminthol. Soc. Wash., 16, 37–73.

Thorne, G., Smolik, J.D. (1971). Identity of *Paratylenchus nanus* Cobb, 1923. Proc. Helminthol. Soc. Wash., 38, 90–99.

Triboy, O., Dragnev, S. (2018). How to use low-productive land for sustainable cultivation of biomass for bioenergy production. Ecol. Pidpriemstv, 7, 55–63 [in Ukrainian].

Van den Berg, E., Tiedt, L.R., Subbotin, S.A. (2014). Morphological and molecular characterisation of several *Paratylenchus* Micoletzky, 1922 (Tylenchida: Paratylenchidae) species from South Africa and USA, together with some taxonomic notes. Nematology, 16, 323–358.

Viketoft, M., Palmborg, C., Sohlenius, B., Huss-Danell, K., Bengtsson, J. (2005). Plant species effects on soil nematode communities in experimental grasslands. Appl. Soil Ecol., 30, 90–103.

Vovlas, N., Troccoli, A., Castillo, P. (1998). Observations on the polinucleate esophageal glands of *Rotylenchus magnus jaeni* Castillo et al., 1994 juveniles with comments on the morphology of a population of *R. agnetis* Szczygiel, 1968 from Italy. Nematropica, 28, 231–240.

Wasilewska, L. (2006). Changes in the structure of the soil nematode community over long-term secondary grassland succession in drained fen peat. Appl. Soil Ecol., 32, 165–179. https://doi.org/10.1016/j.apsoil.2005.07.003

Watson, R.N., Bell, N.L. (2001). Population dynamics of *Paratylenchus nanus* in soil under pasture. 2. Biotic factors and population modelling. Nematology, 3, 255–265.

Winiszewska, G., Dmowska, E., Chałańska, A., Dobosz, R., Kornobis, F., Ilieva-Makulec, K., Skwiercz, A., Ishage, E. (2012). Nematodes associated with plant growth inhibition in Wielkopolska region. J. Plant Prot. Res., 52, 440–446. https://doi.org/10.2478/v10045-012-0071-y

Yuen, P.H. (1964). Four new species of *Helicotylenchus* Steiner (Hopiolaiminae: Tylenchida) and a redescription of *H. canadensis* Waseem, 1961. Nematologica, 10, 373–387.

Yuen, P.H. (1966). The nematode fauna of the regenerated woodland and grassland of Broadbalk Wilderness. Nematologica, 12, 195–214.

Zapałowska, A., Skwiercz, A. (2018). Populations of parasitic nematodes colonizing Jerusalem artichoke (*Helianthus tuberosus* L.). Acta Soc. Bot. Pol., 87, 1–10. https://doi.org/10.5586/asbp.3578