Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Plant-parasitic nematode *Xiphinema parataylori* Lazarova *et al*., 2019 (Nematoda: Longidoridae) from Poland: first report outside the place of species original description

Franciszek Kornobis[1]*, Arnika Przybylska[2]

[1] Department of Entomology and Animal Pests, Institute of Plant Protection – National Research Institute, Poznań, Poland
[2] Department of Molecular Biology and Biotechnology, Institute of Plant Protection – National Research Institute, Poznań, Poland

DOI: 10.24425/jppr.2024.151252

Received: November 15, 2023
Accepted: April 30, 2024
Online publication: August 09, 2024

*Corresponding address:
f.kornobis@iorpib.poznan.pl

Responsible Editor:
Tatyana Stefanovska

Abstract

Plant-parasitic nematodes of the genus *Xiphinema* Cobb, 1913 constitute a group of soil-inhabiting, polyphagous ectoparasites of plant roots, some of which are also vectors of nepoviruses. In this study, 1237 soil samples were taken from different regions of Poland. In two of these samples (0.16% of all collected) *Xiphinema parataylori* Lazarova *et al*., 2019 was present, which constitutes not only the first record from Poland but also the first report outside the Czech Republic and Slovakia, where this species was initially described. Specimens from Poland were largely similar to those from the original description except for a slenderer body (maximal body thickness in two populations found 44.8 and 46.8 vs 54, 55 and 56 µm resulting in higher 'a' index: 50.0 and 50.8 vs 37.8, 40.7 and 41.2). Specimens from the Polish populations were also characterized based on the ITS1 molecular marker. Finally, both populations recorded from Poland were associated with wild pear (*Pyrus pyraster*) extending the number of known host plants.

Keywords: ITS1, molecular markers, morphology, taxonomy, *Xiphinema americanum* sensu lato

Introduction

Plant-parasitic nematodes of the genus *Xiphinema* Cobb, 1913 (family Longidoridae) constitute a group of soil-inhabiting, polyphagous ectoparasites of plant roots. They harm plants either by direct feeding or by the fact that some of these species are vectors of different plant nepoviruses (Taylor and Brown 1997). Currently, over 280 species have been assigned to the genus (Archidona-Yuste *et al*. 2020). Species identification within such a large genus is often difficult. Additionally, the taxonomic status (i.e., species names to which they are assigned) of specimens from populations from a given area sometimes change over time. It is usually associated with new, more accurate observations and/or new research techniques like the usage of molecular markers which are currently a standard part of most taxonomic descriptions (e.g., Rybarczyk-Mydłowska *et al.* 2019; Singh *et al.* 2022). An interesting case study of such a situation involves species *Xiphinema brevicolle* Lordello and Costa, 1961, a species described from Brazil where it was found associated with coffee (Lordello and Da Costa 1961). However, before discussing this species further a nomenclature remark regarding its naming is required to avoid confusion. Luc *et al.* (1998) proposed '*X. brevicollum*' instead of *X. brevicolle*. Later, Monteiro (2010) pointed out that such a change is unacceptable according to the "International Code of the Zoological Nomenclature". Nevertheless, some research papers as well as well as molecular marker descriptions in public databases utilize this incorrect name. Since then, the original description, X. *brevicolle* has been recorded in many countries and continents, including Poland (Szczygieł and Brzeski 1985). Yet, a series of subsequent analyses has indicated that many of these world-distributed

populations represent different species, some of which were formally described (Lazarova *et al.* 2019). For example, populations from the Czech Republic initially reported as *X. brevicolle* (Kumari *et al.* 2005, 2010) together with two additional populations from Slovakia were subsequently described as a new species, *X. parataylori* Lazarova *et al*., 2019 (Lazarova *et al.* 2019), while the range of *X. brevicolle* was regarded to be most likely globally restricted. These findings prompted us to undertake a detailed study of Polish populations morphologically resembling *X. brevicolle*. Thus, this study aimed to provide the first geographic record of *X. parataylori* from Poland and to characterize these populations based on their morphology, morphometrics and ITS1 molecular marker.

Materials and Methods

The study was based on material from 1237 soil samples taken from different regions of Poland and from different host plants. Nematodes were isolated from soil by decanting and sieving (Brown and Boag 1988), however 100 µm mesh for final separation. Subsequently, several specimens for DNA testing were handpicked under the dissecting microscope and preserved in 1M NaCl while the remaining ones were heat-killed and preserved in TAF (Courtney *et al.* 1955). TAF fixed specimens were subsequently transferred to glycerol using the Seinhorst (1959) method, measured and used for morphological study. From the salt-fixed nematodes, four specimens per population were used for extraction of the DNA.

DNA extraction, amplification, and sequence analyses

Genomic DNA from nematodes was extracted using a NucleoSpin Tissue XS kit (Macherey-Nagel) in 50 µl final volume and 1 µl was used as a template for PCR reactions. ITS1 rDNA region was amplified with BL18 (forward) and BV3 (reverse) primers whose sequences and reaction conditions were described by Oliveira *et al.* (2005). PCR products were excised from the gel, eluted using Wizard® SV Gel and PCR Clean-Up System (Promega), and ligated with pJET 1.2 vector (Thermo Fisher Scientific). Plasmid was transformed into *Escherichia coli* DH10B strain, amplified, isolated from bacteria cells using NucleoSpin Plasmid Kit (Macherey-Nagel), and sequenced. The obtained chromatograms were visualized using Chromas and subsequently assembled in BioEdit software (Hall 1999). Obtained sequences were BLAST searched to check their nematode origin and the phylogenetic tree was created using MEGA11:Molecular Evolutionary Genetics Analysis version 11 software (Tamura *et al.* 2021) using Maximum Likelihood Method and Tamura-Nei model (Tamura and Nei 1993). Initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. Sequences used to create a tree are listed in Table 1.

Table 1. Sequences from selected *Xiphinema* populations used to create a phylogenetic tree with their accession number and place of origin

Nematode species	Accession number	Origin
X. parataylori	OR777149	Poland
X. parataylori	OR777150	Poland
X. parataylori	MH248808	Slovakia
X. parataylori	MH248807	Slovakia
X. brevicollum	HQ184474	Japan
X. brevicollum	AY430190	South Africa
X. brevicollum	AY430181	Brazil
X. brevicollum	JX218046	China
X. diffusum	AY359858	Taiwan
X. diffusum	MK050006	South Korea
X. americanum	JN091971	Japan
X. inaequale	HM163203	India
X. incognitum	AY359857	Taiwan

Results

Two samples (0.16% of all collected) contained *Xiphinema parataylori*. One nematode population was found near the town of Borkowice (N50.7423; E17.7213) and a second near Klucze (N50.3365; E19.5518) (Fig. 1). The host plant for both nematode populations was wild pear (*Pyrus pyraster*). The obtained ITS1 sequences differed one from another in one nucleotide. When subjected to BLAST search in GenBank both obtained sequences showed 99.55% similarity to sequence MH248808 obtained from *X. parataylori* and published by Lazarova *et al.* (2019). Data on the morphometry of the analyzed populations are presented in Table 2. Photographs illustrating their morphology are found in Figure 2.

PCR reactions and sequence analyses

As a result of PCR reactions products with size 468 bp were observed in samples derived from both analyzed populations (Fig. 3A, B). Sequences differed in one nucleotide. When subjected to BLAST search

Table 2. Morphometrics of *Xiphinema parataylori* populations from Poland

Trait	Population from Klucze (N50.3365; E19.5518)	Population from Borkowice (N50.7423; E17.7213)				
		Females *n* = 20	J1 *n* = 3	J2 *n* = 6	J3 *n* = 7	J4 *n* = 8
L	2279 ± 93.6 (2056–2405)	2338 ± 114.6 (2126–2670)	840 (808–862)	1013 ± 52.9 (949–1104)	1349.1 ± 96.66 (1256–1538)	1870 ± 151.6 (1645–2137)
a	50.8 ± 1.43 (49–53)	50.0 ± 2.97 (43–56)	37.7 (35–40)	41.9 ± 2.63 (38–45)	43.4 ± 2.19 (40–46)	46.1 ± 1.51 (43–47)
b	6.6 ± 0.43 (5.9–7.4)	7.0 ± 0.63 (6.1–8.2)	4.5 (4.3– 4.3)	4.5 ± 0.29 (4.2–4.8)	5.0 ± 0.48 (4.4–5.8)	5.9 ± 0.55 (4.9–6.6)
c	75.9 ± 5.13 (66–83)	80.0 ± 5.24 (72–90)	23.1 (23–23)	29.2 ± 1.62 (28–32)	39.0 ± 1.93 (35–40)	56.1 ± 4.71 (48–61)
c′	0.98 ± 0.05 (0.87–1.03)	0.98 ± 0.07 (0.87–1.1)	2.49 (2.31–2.64)	2.05 ± 0.11 (1.94–2.27)	1.64 ± 0.1 (1.5–1.8)	1.24 ± 0.10 (1.1–1.33)
V/ replacement odontostylet	50.1 ± 0.88 (48–51)	51.0 ± 2.07 (49–56)	56.0 (55–57)	67.3 ± 2.07 (64–70)	79.9 ± 1.77 (77–82)	96.8 ± 3.01 (91–100)
Odontostylet length	95.5 ± 2.02 (93–101)	96.1 ± 2.25 (91–100)	48.0 (46–49)	55.5 ± 0.55 (55–56)	67.3 ± 1.38 (65–69)	80.4 ± 3.29 (76–87)
Odontophore length	54.6 ± 2.57 (51–58)	54.4 ± 2.64 (49–58)	34.0 (33–36)	37.3 ± 1.21 (35–38)	40.7 ± 2.25 (39–45)	49.3 ± 2.14 (47–53)
Width at lips	14.3 ± 0.62 (13–15)	13.8 ± 0.52 (13–15)	9.0 (9–9)	10.2 ± 0.41 (10–11)	10.9 ± 0.38 (10–11)	12.2 ± 0.46 (12–13)
Width at vulva/ midbody	44.8 ± 1.85 (42–47)	46.8 ± 1.85 (44–50)	22.3 (21–23)	24.3 ± 2.66 (21–29)	31.1 ± 2.30 (32–38)	40.6 ± 3.38 (35–45)
Width at anus	30.8 ± 1.29 (29–33)	29.9 ± 1.29 (27–33)	14.7 (14–16)	17.0 ± 1.41 (15–19)	21.7 ± 1.80 (20–25)	27.1 ± 2.59 (24–31)
Tail length	30.1 ± 1.51 (28–34)	29.4 ± 2.3 (26–34)	36.3 (35–37)	34.8 ± 2.79 (31–39)	35.6 ± 2.30 (32–38)	33.4 ± 1.92 (30–36)
Hyaline part of tail length	9.4 ± 0.84 (8–10)	9.7 ± 1.08 (8–12)	7 (6–8)	6.9 ± 0.98 (5–8)	8.0 ± 1.15 (7–10)	8.2 ± 0.89 (7–9)

All data are in µm and in form: mean ± standard deviation (mean). Standard deviation is not given if there are less than 5 measurements

Fig. 1. A map presenting the distribution of *Xiphinema parataylori* populations from Poland

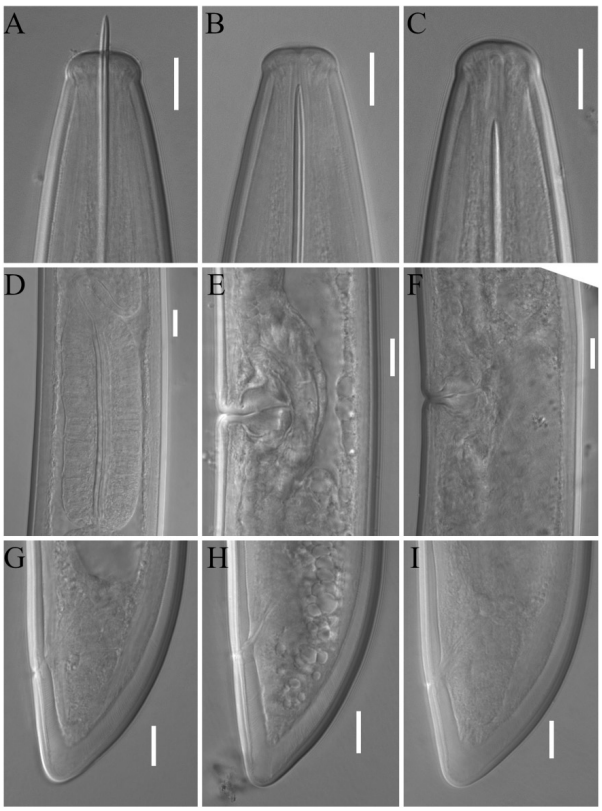
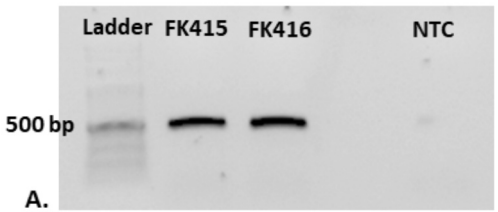


Fig. 2. Morphology of *Xiphinema parataylori* female: A–C – anterior body end; D – pharyngeal bulb; E, F – vagina; G–I – tails



Seq->	FK415	FK416	X.p.	X.b.
FK415	ID	1,000	0,993	0,973
FK416	1,000	ID	0,993	0,973
X.p.	0,993	0,993	ID	0,975
X.b.	0,973	0,973	0,975	ID

B.

Fig. 3. Analysis of ITS1 region from *Xiphinema parataylori* populations. A – results of PCR reactions with primers amplifying part of ITS1 region; B – results of comparative sequence analysis between nucleotide sequences encoding ITS1 region in two *X. parataylori* analyzed populations and reference sequences. FK415 – Borkowice population; FK416 – Klucze population; NTC – no template control; X.p. – reference for *X. parataylori*; X.b. – reference for *X. brevicollum*

in GenBank, both obtained sequences revealed 99.55% similarity to sequence MH248808.1 from *X. parataylori* obtained by Lazarova *et al.* (2019). Obtained sequences were deposited in GenBank under accession numbers: OR777149 sequence from the Borkowice

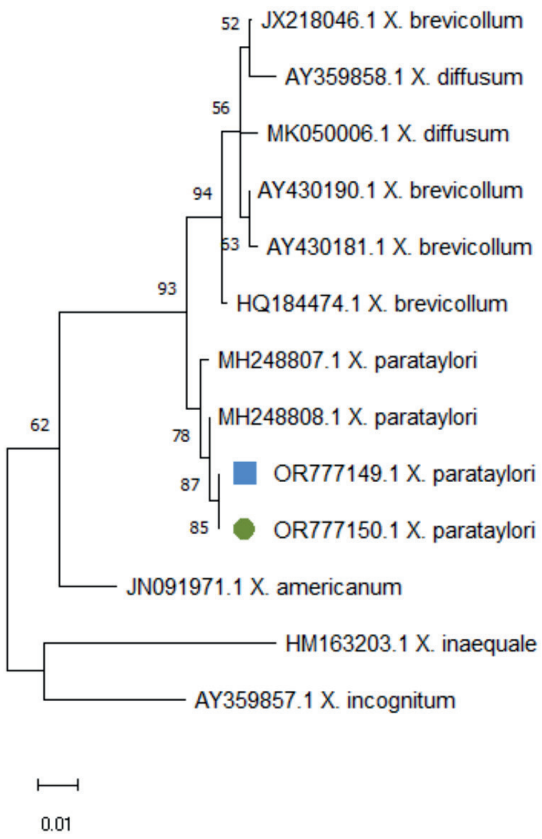


Fig. 4. A maximum likelihood phylogenetic tree based on ITS1 marker with two analyzed *Xiphinema parataylori* populations and reference sequences from GenBank. OR777149.1 – Borkowice population; OR777150.1 – Klucze population

population and OR777150 from Klucze. The evolutionary analysis, illustrated in the phylogenetic tree (Fig. 4) confirmed that populations analyzed in this study cluster with *X. parataylori*.

Discussion

To the best of our knowledge, the present report of *X. parataylori* constitutes the first record of this species outside the Czech Republic and Slovakia where it was first described (Lazarova *et al.* 2019). In terms of their general morphology specimens from Poland are largely similar to the ones from the original description (Lazarova *et al.* 2019) except for maximal body thickness. The mean values of this parameter in specimens from three specimens described from the Czech Republic are 54, 55 and 56 µm, while in specimens from Poland, it is 44.8 and 46.8 (Tab. 2). The difference in body thickness results also had a lower 'a' index in populations from the Czech Republic: means 41.2, 37.8 and 40.7 versus 50.8 and 50.0. However, the remaining data regarding morphology, morphometrics

as well as data from the ITS1 marker showed consistency with those given by Lazarova *et al*. 2019 (Tab. 2; Figs. 2 and 4). The reason(s) for the observed difference may be due to varying parasitism on different host plants, inter-populational variability or differences in microscopic slide preparation. The finding of *X. parataylori* in Poland raises also a question regarding the validity of the previous record of *X. brevicolle* from Poland (Szczygieł and Brzeski 1985). Unfortunately, any proof materials (e.g., microscopic slides with the specimens, drawings or measurements) complementing that paper are not available. However, following the data provided by Lazarova *et al*. 2019 it is likely that these populations represent another, morphologically similar species, perhaps *X. parataylor*i. In previous records from Czech Republic and Slovakia, *X. parataylori* was found to be associated with grapevine, peach and tetterwort (Kumari *et al*. 2005, 2010; Lazarova *et al.* 2019). The current finding of this species on wild pear extends the number of known host plants.

References

Archidona-Yuste A., Cai R., Cantalapiedra-Navarrete C., Carreira J.A., Rey A., Viñegla B., Liébanas G., Palomares-Rius J.E., Castillo P. 2020. Morphostatic speciation within the Dagger Nematode *Xiphinema hispanum*-Complex Species (Nematoda: Longidoridae). *Plants* 9 (12): 1649. DOI: https://doi.org/10.3390/plants9121649

Brown D.J.F., Boag B. 1988. An examination of methods used to extract virus vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. Nematologia Mediterranea 16 (1): 93–99.

Courtney W.D., Polley D., Miller V.L. 1955. TAF, an improved fixative in nematode technique. Plant Disease Reptort 39: 570–571.

Hall T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Kumari S., Decraemer W., De Luca F. 2010. Molecular characterization of *Xiphinema brevicollum* (Nematoda: Longidoridae) from the Czech Republic. European Journal of Plant Pathology 128 (2): 243–250. DOI: https://doi.org/10.1007/s10658-010-9651-8

Kumari S., Polák J., Choutka R. 2005. Plant-parasitic nematodes of the genus *Xiphinema* (Nematoda: Longidoridae) in the vineyards of the Czech Republic. Nematology 7 (1): 81–93. DOI: https://doi.org/10.1163/1568541054192117

Lazarova S., Oliveira C. M. G., Prior T., Peneva V., Kumari S. 2019. An integrative approach to the study of *Xiphinema brevicolle* Lordello and Da Costa 1961, supports its limited distribution worldwide (Nematoda: Longidoridae). European Journal of Plant Pathology 153: 441–464. DOI: https://doi.org/10.1007/s10658-018-1571-z

Lordello L.G. E., Costa C.P. 1961. A new nematode parasite of coffee roots in Brazil. Revista Brasileira de Biologia 21: 363–366.

Luc M., Coomans A., Loof P.A., Baujard P. 1998. The *Xiphinema* americanum-group (Nematoda: Longidoridae). 2. Observations on *Xiphinema brevicollum* Lordello & da Costa, 1961 and comments on the group. Fundamental and Applied Nematology 21 (5): 475–490.

Monteiro A.R. 2010. Nomenclatorial note on *Xiphinema brevicolle* Lordello & Costa, 1961 (Nematoda: Longidoridae). Nematologia Brasileira 34: 1–2.

Oliveira C.M., Fenton B., Malloch G., Brown D.J., Neilson R. 2005. Development of species-specific primers for the ectoparasitic nematode species *Xiphinema brevicolle*, *X. diffusum*, *X. elongatum*, *X. ifacolum* and *X. longicaudatum* (Nematoda: Longidoridae) based on ribosomal DNA sequences. Annals of Applied Biology 146 (3): 281–288.

Rybarczyk-Mydłowska K., Dmowska E., Kowalewska K. 2019. Phylogenetic studies on three *Helicotylenchus* species based on 28S rDNA and mtCOI sequence data. Journal of Nematology 51: e2019-33. DOI: https://doi.org/10.21307/jofnem-2019-033

Seinhorst J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. Nematologica 4: 67–69.

Singh P. R., van de Vossenberg B., Rybarczyk-Mydłowska K., Kowalewska-Groszkowska M., Bert W., Karssen G. 2022. An integrated approach for synonymization of *Rotylenchus rhomboides* with *R. goodeyi* (Nematoda: Hoplolaimidae) reveals high intraspecific mitogenomic variation. Phytopathology 112 (5): 1152–1164. DOI: https://doi.org/10.1094/PHYTO-08-21-0363-R

Szczygieł A., Brzeski M.W. 1985. Distribution of Longidoridae, Xiphinemidae and Trichodoridae. In: "Atlas of Plant Parasitic Nematodes of Poland" (Alphey T.J.W., ed.). Scottish Crop Research Institute, Dundee, Scotland, 32 pp.

Tamura K., Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512–526.

Tamura K., Stecher G., Kumar S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38: 3022–3027. DOI: https://doi.org/10.1093/molbev/msab120

Taylor C.E., Brown D.J.F. 1997. Nematode Vectors of Plant Viruses. CAB International, Wallingford, UK, 286 pp.