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Molecular characterization of root-lesion nematode, *Pratylenchus* species, and their prevalence in New Zealand maize fields

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Abstract

Root-lesion nematodes (*Pratylenchus* spp.) are significant plant parasites, causing substantial crop damage worldwide. This study aimed to characterize *Pratylenchus* spp. in New Zealand maize fields using molecular techniques and map their prevalence. Soil sampling from 24 maize fields across the North and South Islands provided 381 composite samples. Root-lesion nematodes were extracted using the sieving-centrifugal-sugar flotation method and differentiated into five morphospecies. Molecular characterization involved direct partial sequencing of the D2/D3 28S rDNA, ITS rDNA, and COX1 mtDNA regions using Sanger technology from a single nematode. Five *Pratylenchus* species were identified: *P. neglectus*, *P. crenatus*, *P. thornei*, *P. penetrans*, and *P. pratensis*, confirmed by phylogenetic analysis. Prevalence mapping showed *P. neglectus* and *P. crenatus* in all sampled fields, while *P. thornei*, *P. penetrans*, and *P. pratensis* were more localized. This study is the first to report these *Pratylenchus* species on maize in New Zealand and provides the first partial sequences of the D2/D3, COX1, and ITS regions for these species on maize in New Zealand. The findings highlight the diversity of *Pratylenchus* populations in New Zealand maize fields and emphasize the need for region-specific management strategies to mitigate crop damage.

Impact Statement

This is the first comprehensive study to investigate the prevalence and identification of *Pratylenchus* spp. in New Zealand, using molecular characterization techniques. Despite reports of seven *Pratylenchus* spp. in various crops, no studies in New Zealand have used a molecular approach for its identification. Additionally, *Pratylenchus* spp. association in maize have not been examined. This study provides information for future research exploring the impact of *Pratylenchus* spp. on the production and sustainability of maize and offers information for future nematode management strategies.

Introduction

Root-lesion nematodes, belonging to the genus *Pratylenchus* Filipjev [1936], are recognized as important plant-parasitic nematodes (PPN) worldwide, causing considerable damage up to 20%–50% in cereal crops, including wheat and maize (Thompson et al. [2008], Thompson et al. [2009]). *Pratylenchus* spp. has a wide host range, including vegetables, beans, tubers, and orchard crops (Castillo and Vovlas [2007], Chowdhury et al. [2022]). The *Pratylenchus* genus comprises over 100 species, with *P. neglectus* being the most distributed and economically damaging species (Castillo and Vovlas [2007], Kumari [2015]). *Pratylenchus* spp. are migratory endoparasites that cause the third most destruction of crops after root-knot and cyst nematodes. *Pratylenchus* spp. occur in a wide range of climate zones, including tropical, subtropical, and temperate environments (Castillo and Vovlas [2007], Jones et al. [2013], Divsalar et al. [2018]). The negative impact of *P. neglectus* (Rensch [1924]) Filipjev and Schuurmans Stekhoven [1941], *P. pene-*

trans (Cobb [1917]) Filipjev and Schuurmans Stekhoven [1941], and *P. thornei* Sher and Allen [1953] was documented in many regions such as in Australia, the USA, Africa, and Asia (Riga et al. [2008], Yan et al. [2008], Thompson et al. [2016]). Recent studies have described several new *Pratylenchus* species, highlighting the ongoing progression of identifying their diversity. For example, researchers identified *P. haiduongensis* in Vietnam (Nguyen et al. [2017]), *P. rwandae* in Rwanda (Singh et al. [2018]), and *P. dakotaensis* in the USA (Handoo et al. [2021]).

Pratylenchus spp. host selectivity and severity vary depending on the species present (Castillo and Vovlas [2007]). Therefore, it requires the right identification methods for precise identification to species level, which is crucial for managing nematodes. Morphological variations among *Pratylenchus* species have been well-documented within and between species (Handoo and Golden [1989], Castillo and Vovlas [2007]). Morphological identification can be difficult and time-consuming for this genus (Kumari [2015]). Additionally, mor-

phological attributes may also be altered due to variations in geographical location, host plant, nutrition, and other environmental factors (Bogale et al. 2020).

The taxonomy of the genus has been a subject of ongoing research and there are pitfalls in *Pratylenchus* species-level identification reported (Janssen et al. 2017b). However, Janssen et al. (2017b) reported that *Pratylenchus* species. identification based solely on morphology is inconclusive for this genus. Additionally, having a multi-gene phylogeny of using nuclear ribosomal and mitochondrial gene sequences can provide reliable identification of the species belonging to this genus (Kumari 2015, Bogale et al. 2020, Handoo et al. 2021). The use of both morphological and molecular techniques has become essential for the accurate identification of species in the *Pratylenchus* genus (Subbotin et al. 2008, Divsalar et al. 2018, Nguyen et al. 2023). Most studies indicated that morphological identification coupled with sequencing of two or more genes' regions could be reliable, particularly for the *Pratylenchus* species (Janssen et al. 2017a, Handoo et al. 2021).

Previous studies have shown that DNA sequencing, especially the 28S D2/D3 rDNA, ITS, and COI (COX1) mtDNA fragments, is effective for characterizing *Pratylenchus* populations and conducting phylogenetic analyses (Subbotin et al. 2008, Kumari 2015, Janssen et al. 2017a,b, Handoo et al. 2021). Therefore, *Pratylenchus* species in this study were identified by amplifying three gene regions: COX1 mtDNA, LSU D2/D3 28S rDNA, and ITS rDNA, and were compared with the morphological characters reported. Recent surveys in New Zealand indicate a higher abundance of *Pratylenchus* spp. in maize and wheat fields; however, these surveys lack a detailed characterization of these nematodes (Thiellier and Kularathna 2023, Thiruchchelvan et al. 2023). Therefore, the present study aimed to (1) characterize the *Pratylenchus* spp. based on the molecular analysis and (2) map the species prevalence in New Zealand maize.

Materials and methods

Soil sampling and extraction of *Pratylenchus* species

During June–November 2022, soil sampling was conducted in maize-growing regions of New Zealand. A total of 381 composite soil samples were collected from 24 fields across three regions including Canterbury (South Island), Waikato, and Manawatu-Whanganui (North Island). Fields were divided into 2 or 3 blocks (>5 ha 3 blocks) based on size, with 10–25 samples per field collected in a double or triple zigzag pattern. At each sampling point, 15 soil cores (30 cm deep, 2 cm diameter) were taken using a soil sampler (OAK-FIELD Apparatus, USA) within a 4 m radius, averaging 750 g of soil per sample. From these, 24 composite samples were created for *Pratylenchus* spp. extraction by mixing ∼75–100 g subsamples from each field sample, resulting in ∼1–2 kg of composite soil per field. The sieving-centrifugal-sugar flotation method (Jenkins 1964) was used for nematode extraction. A 100 g of soil was mixed with 1 l of tap water. The soil was settled, and the supernatant was passed through nested sieves (150-μm and 38-μm apertures, Glenammer, UK). The process was repeated thrice, and the soil from the 38-μm sieve was centrifuged at 576 *g* for 5 minutes. The supernatant was discarded, and the pellet was

mixed with 45% (w/v) sucrose solution before being centrifuged for 1 minute at 576 g. The supernatant was then sieved (38-μm aperture), washed, and the nematodes were collected into 50 ml specimen bottles. The samples were stored at 4°C for morphological identification (Kularathna et al. 2019).

Morphospecies differentiation

Pratylenchus spp. were identified to genus level based on morphological characteristics such as head shape, stylet and stylet knob shape, pharynx, gland lobe length, female tail terminus, and position of vulva (V-value) (Fortuner 1988, Handoo and Golden 1989, Mai et al. 1996). Nematodes were heat-killed on a glass slide, observed at 40×, 100×, and/or 600× magnification using an inverted compound light microscope (Olympus-CKX53, Japan). Morphometric measurements were recorded for females and available males using the Olympus cellSens Entry system as per De Man Formulae (Nemaplex 2019). Five morphospecies were differentiated with observed morphological characteristics as shown in Figs 1, 2, and Table 1. Each morphospecies was identified at the species level using the molecular analysis described below.

Molecular identification: DNA extraction, PCR, and phylogenetic analysis

Genomic DNA was extracted from individual nematodes using the worm lysis buffer method (Chowdhury et al. 2020, Handoo et al. 2021). Female nematodes were initially washed in double distilled water (ddH$_2$O) on a glass slide, followed by surface sterilization in 1% NaOCl, and then subjected to three additional washes with ddH$_2$O. The sterilized nematodes were chopped in a cavity slide with a sterile surgical blade and transferred to a centrifuge tube containing worm lysis buffer (2 μl Proteinase K enzyme (600 μg ml^{-1}), 2 μl PCR buffer with MgCl$_2$ (10×), and 6 μl Millipore water). The tubes were incubated at −20°C for 30 minutes, followed by 65°C for 1 hour and 95°C for 10 minutes to terminate the reaction. The extracted DNA was either used immediately for PCR or stored at −20°C (Huang and Yan 2017).

PCR amplification targeted D2/D3 fragments of 28S rDNA, ITS rDNA, and COX1 gene from mtDNA (Handoo et al. 2021) using primers D2A/D3B, ITS5/ITS4, and JB3/JB4.5 (Supplementary Table S1). Reactions were carried out in 20 μl volumes, consisting of 2 μl DNA template, 1 μl each of forward and reverse primers (10 μmol l^{-1}), 10 μl Dream-Taq Green PCR Master Mix (2×), and 6 μl Millipore water. Thermocycling conditions were as follows: for D2A/D3B and ITS5/ITS4 primers, initial denaturing at 95°C for 3 minutes, followed by 30 cycles of 95°C for 30 seconds, annealing at 52°C (D2A/D3B) or 55°C (ITS4/ITS5) for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes (Subbotin et al. 2006). For the COX1 primers JB3/JB4.5, conditions included an initial denaturation at 95°C for 5 minutes, followed by 5 cycles of 95°C for 30 seconds, 54°C decreasing by 1°C per cycle for 30 seconds, 72°C for 30 seconds, and 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 10 minutes (Bowles et al. 1992, Derycke et al. 2010). PCR products were confirmed by electrophoresis on a 1% agarose gel stained with GelRed, visualized, using the Gel-Doc Go imaging system (Bio-Rad). The amplified DNA was sequenced at the Lincoln University sequencing facility using

Sanger dideoxy sequencing technology (Applied Biosystems, HITACHI, 3500 XL, Genetic Analyzer, New Zealand).

Consensus sequences were generated using forward and reverse sequences in Geneious Prime 2023.2.1. Sequences were deposited in GenBank if they had over 98% similarity and 90% query coverage during blasting. Phylogenetic trees were constructed using Bayesian inference with MAFFT multiple sequence alignment (Katoh and Standley 2013) and MrBayes v.3.2.6 (Ronquist et al. 2012), selecting the GTR + G model for COX1 and ITS and GTR + G + I model for LSU D2/D3 sequences based on jModelTest (Posada 2008, Darriba et al. 2012). Bayesian analysis involved 4 Markov chains for 10^6 generations, sampling every 200 generations, with burn-in samples discarded to generate a 50% majority rule consensus tree (Xia et al. 2021).

Prevalence of *Pratylenchus* sp. in New Zealand maize fields

The prevalence of the five different species of *Pratylenchus* sp. in the presence or absence data matrix was mapped using ArcGIS (ArcMap) 10.8.1 software.

Results and discussion

Molecular identification and phylogenetic analysis

This study focused on the molecular identification of five morphospecies belonging to the *Pratylenchus* genus collected from New Zealand maize fields and was complemented by some morphological observations. Using the LSU and COX1 gene regions, the molecular data strongly supported the identification of *Pratylenchus neglectus*, *P. crenatus* Loof (1960), *P. thornei*, *and P. penetrans*. The identified *Pratylenchus* morphospecies showed sequence similarities ranging from 98% to 100% when compared to species in the NCBI database. The D2/D3 sequences for all five species showed 98%–100% similarity, while the COX1 sequences for four species exhibited 99%–100% similarity. The COX1 region of *P. pratensis* was not successfully amplified, and ITS fragment amplifications were only successful for *P. thornei, P. neglectus*, and *P. pratensis* De Man 1880. The poor-quality sequences obtained for *P. penetrans* and *P. crenatus* in the ITS region may be attributed to several factors. One possible reason is the lack of primer specificity, as the primers used may not have been sufficiently optimized for these nematode species (Blok and Powers 2009). Additionally, background noise or contamination could have contributed to the poor-quality sequences, potentially due to the co-amplification of non-target DNA, as reported by De Ley et al. (2002).

The best sequence match for each identified species is detailed in Supplementary Table S2, while the deposited sequences in GenBank, along with the generated accession numbers, are listed in Supplementary Table S3. Phylogenetic trees generated from LSU D2/D3 28S rDNA (Fig. 3) include 70 ingroups and an outgroup taxon; the ITS rDNA tree (Supplementary Fig. S1) includes 24 ingroups and an outgroup taxon; and the COX1 mtDNA tree (Fig. 4) comprises 72 ingroups and an outgroup taxon. All three trees indicate that the newly identified *Pratylenchus* species are distinct from one another, each forming 100% supported clades with the respective species already available in the database.

The amplification success and sequence similarities observed in this study are consistent with findings from other nematode research, where the D2/D3 28S rDNA and COX1 mtDNA regions are commonly used for species identification and phylogenetic analysis (Holterman et al. 2009, Janssen et al. 2017a,b, Handoo et al. 2021). However, the ITS region has been reported to be more variable, with amplification success often depending on the species and the quality of the extracted DNA (Powers et al. 2011). In comparison, other studies using different primers or alternative regions, such as 18S rDNA, have reported varying degrees of success in amplifying ITS regions (Subbotin et al. 2001, Subbotin et al. 2023).

Some minor morphometric deviations were observed compared to type populations, highlighting the potential influence of geographic and environmental factors on nematode morphology (Bogale et al. 2020). Morphospecies 1 (*P. neglectus*): molecular analysis confirmed the identity of *P. neglectus*. Morphologically, the New Zealand population exhibited minor variations in body length and tail shape, but these differences fall within the expected range for this species (Supplementary Table S4). Similar to the findings of Handoo and Golden (1989) and Xia et al. (2021), these minor deviations suggest that environmental conditions or local adaptation may influence certain morphological traits (Bogale et al. 2020). Morphospecies 2 (*P. crenatus*): the molecular data matched with *P. crenatus* sequences in the NCBI database; however, minor morphometric discrepancies were noted, particularly in stylet length (Supplementary Table S5). The New Zealand population showed slightly longer stylets (17.1–18.7 μm) compared to previous reports, such as Kumari (2015) (13–15 μm) but aligned with Loof (1960) (14–18 μm). These differences could be due to natural variability within the species or local adaptations in New Zealand's environmental conditions as reported by Bogale et al. (2020). Morphospecies 3 (*P. thornei*): molecular identification of *P. thornei* was confirmed by the gene sequencing. While most morphological traits aligned with known descriptions (Handoo and Golden 1989, Movahedifar and Azimi 2020), slight differences in stylet length were observed. These minor discrepancies are not uncommon and have been previously noted in studies of global populations (Fayazi et al. 2012) (Supplementary Table S6). The observed variations may be influenced by factors such as sampling location, host plant differences, or local environmental conditions as suggested by Fayazi et al. (2012). Morphospecies 4 (*P. penetrans*): both molecular and morphological data confirmed the identification of *P. penetrans* in the New Zealand populations. The morphometric traits were consistent with previous descriptions (Bogale et al. 2021, Gil et al. 2021). Although slight variations in stylet length were recorded in the New Zealand populations, these differences are within an acceptable range and are not expected to impact species identification (Supplementary Tables S7 and S8).

Morphospecies 5 (*P. pratensis*): The population of *P. pratensis* described in this study shares several key morphological and molecular features with the populations reported by Janssen et al. (2017a) (Supplementary Tables S9 and S10). Morphometrically, the individuals in our study exhibit a body length of 458–556 μm in females and 469–512.9 μm in males, with lip regions containing three annules and well-separated basal knobs on a robust stylet. The vulva in females is located at 77.6%–80.9% of the body length, and the tail tips are symmetrically conoid, with lengths ranging from 20 to 26.6 μm. Males have spicules that are curved, measuring 14.4–19.3 μm in length, with a bursa enveloping the tail. However, a slight
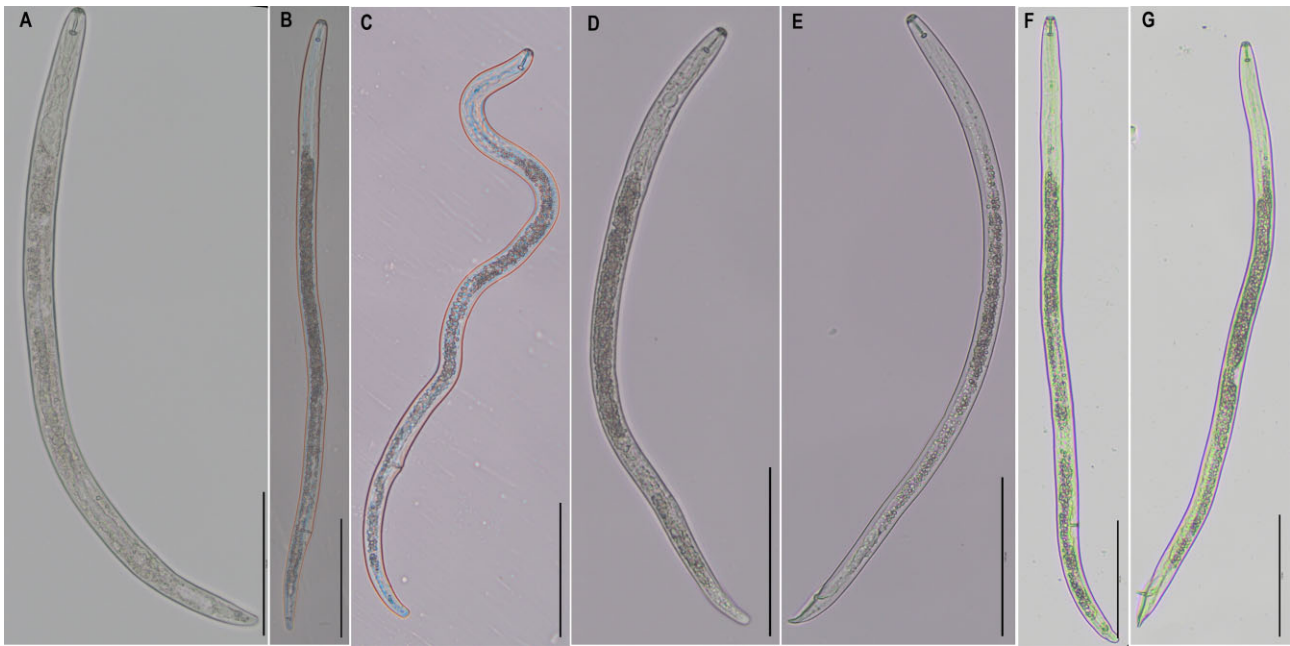
Figure 1. Photomicrographs of the *Pratylenchus* spp. identified in New Zealand maize fields; (A) heat-killed female of morphospecies 1 (slightly ventrally curved); (B) heat-killed female of morphospecies 2 (almost straight); (C) live female of morphospecies 3 (heat-killed females are C shape); (D) heat-killed female of morphospecies 4 (moderately slender, or straight); (E) heat-killed male of morphospecies 4 (moderately slender, or straight); (F) heat-killed female of morphospecies 5 (almost straight); and (G) heat-killed male of morphospecies 5 (almost straight) (scale bars = 100 μm).

Table 1. Morphological characteristics used for the differentiation of morphospecies of the *Pratylenchus* spp. in New Zealand maize fields.

Characteristics	Morphospecies 1	Morphospecies 2	Morphospecies 3	Morphospecies 4	Morphospecies 5
n	10	10	7	9	8
Body length (μm)	474.0 (402.4–556.4)	454.0 (382.8–514.9)	526.1 (503.6–548.9)	417.4 (393.8–435.4)	531.2 (458.8–556.4)
a (L/W)	23.8 (19.7–28.8)	24.7 (21.6–27.7)	26.6 (25.2–28.4)	23.6 (19.9–25)	25.2 (22–29)
b (L/I)	4.3 (3.5–4.9)	4.3 (3.7–4.6)	4.7 (4.7–4.8)	4.2 (3.9–4.5)	4.3 (3.5–5.2)
c (L/T)	19.7 (16.7–22.1)	20.7 (19–22.2)	20.5 (19.3–21.7)	16.6 (13.6–20.8)	22.3 (20.1–25.5)
c' (T/ABD)	1.9 (1.4–2.5)	2.0 (1.7–2.3)	2.3 (2.2–2.5)	2.3 (1.8–2.8)	1.9 (1.3–2.4)
V% (V/L *100)	79.8 (72–82.6)	81.2 (76.6–83.5)	76.4 (75.6–77.4)	78.2 (76.4–80)	79.1 (77.6–80.9)
Stylet (μm)	17.6 (15.8–19.7)	17.7 (17.1–18.7)	18.6 (18–19.2)	16.6 (15.7–17.5)	17.8 (16.2–19.9)
Heat-killed female	Slightly ventrally curved or straight	Moderately ventrally curved or straight	C-shaped curved	Moderately slender, or straight	Almost straight
Lip annuli	Two	Three	Three	Three	Three
Stylet knob	Rounded, anteriorly intended	Rounded	Broadly rounded, anteriorly flattened	Broadly rounded	Well-separated
Tail terminus	Round, no annulation	Crenate with annulation	Truncated, phasmid in mid tails	Rounded with a smooth tip	Symmetrically conoid
Males	Absence	Absence	Absence	Presence	Presence
Spicule	-	-	-	16.5–16.9	14.4–19.3

n- number of nematodes measured; L- body length, W- maximum body diameter; I- intestine length from the anterior end; T-tail length; ABD- body diameter at anus; V- distance to vulva from the anterior end

variation is observed in the stylet length of the specimens examined. The stylet length in females from New Zealand population ranges from 16 to 19.9 μm, and in males, it ranges from 15.8 to 17.1 μm. This contrasts with the type locality of *P. pratensis* in The Netherlands, where the stylet length is consistently 14–15 μm (Loof 1974 in CIH Descriptions of PPNs, 4, # 52). The stylet length in *Pratylenchus* species has been noted as a stable morphological character (Roman and Hirschmann 1969, Janssen et al. 2017a). The larger stylet measurements observed in the New Zealand specimens, therefore, are unlikely to be the result of environmental variability. This suggests that the population may represent a distinct morphological variant within the known species boundaries. Given that

species such as *P. penetrans* and *P. pratensis* are currently regarded as species complexes, it is probable that the specimens encountered in this study belong to cryptic species within these complexes. These differences in stylet length, along with variations in tail morphology, suggest that while the population in our study falls within the *P. pratensis* species complex, it may represent a distinct taxon within this group. Future studies need to be done to clarify this issue.

Additionally, the ecological context of the populations described in this study differs from that of the original type locality of *P. pratensis*, which is typically found in moist meadows or swampy areas of Europe (Loof 1960). In contrast, the specimens examined in this study were collected from
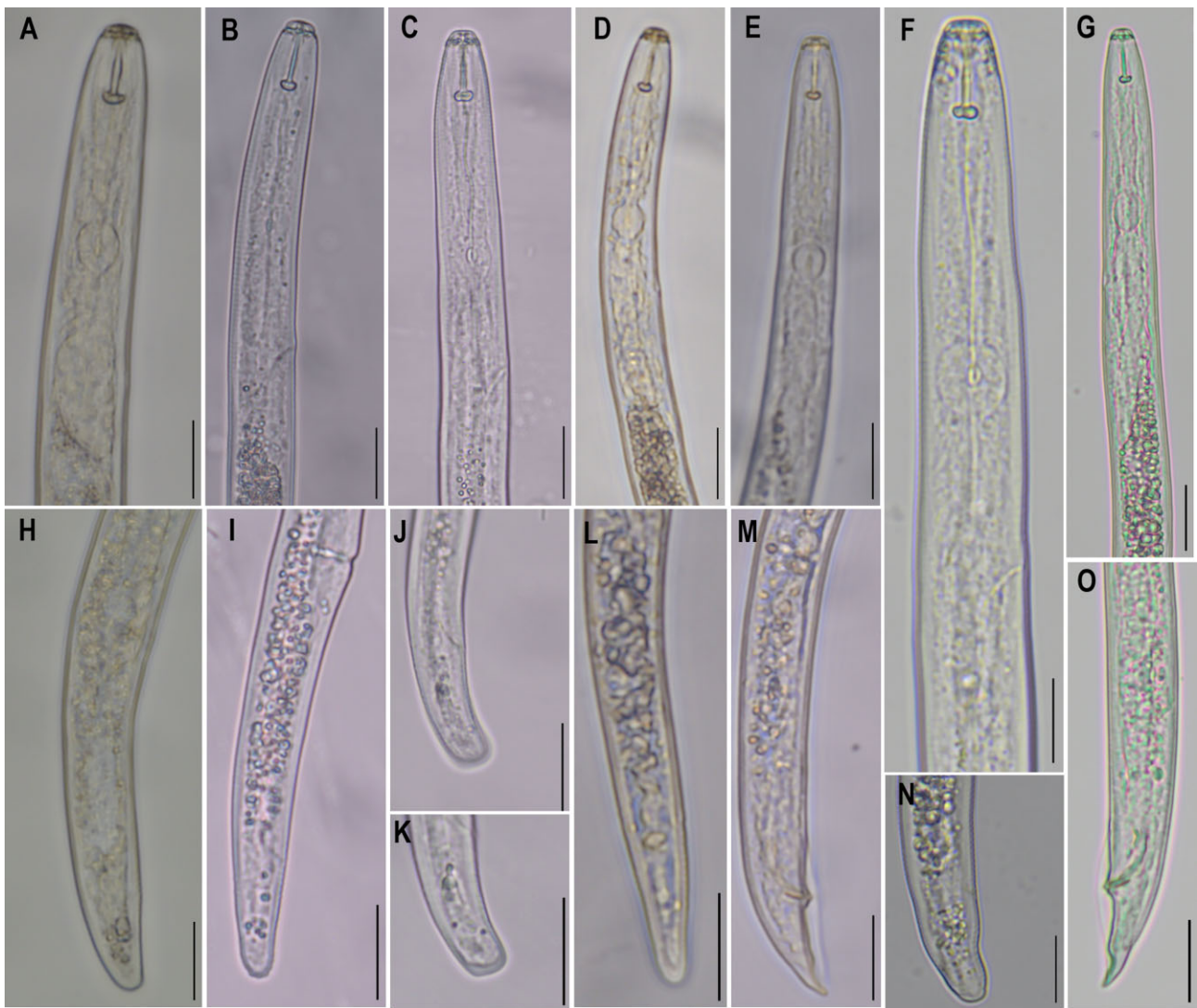
Figure 2. Photomicrographs of the *Pratylenchus* spp. identified in New Zealand maize fields; A-anterior part (rounded stylet knobs indented on the anterior surfaces), H-posterior part (rounded tail terminus has no annulation) of morphospecies 1 female; B-anterior part (rounded stylet knobs), I-posterior part (crenated tail terminus with annulation) of morphospecies 2 female; C-anterior part (broadly rounded almost anteriorly flattened basal knob), J, K-posterior part (slightly conical towards the end and truncated terminus). A phasmid in mid-tail of morphospecies 3 female; D-anterior part (basal knob broadly rounded), L-posterior part (tail is a long-rounded shape with a smooth tip) of morphospecies 4 (female); E-anterior part (basal knob broadly rounded), M-posterior part (with bursa irregularly crenate and enveloping tail tip) of morphospecies 4 male; F-anterior part (well-separated basal knobs), N-posterior part (symmetrically conoid tail terminus) of morphospecies 5 female; and G-anterior part (well-separated basal knobs.), O-posterior part (bursa enveloping the tail) of morphospecies 5 male (scale bars = 20 μm).

cultivated maize fields, suggesting potential ecological divergence. Given the rarity of *P. pratensis* in Europe and Asia and its typical association with wild grasses in natural environments, the population described here might be more closely related to or represent another species within the *P. pratensis* complex.

The molecular evidence from the LSU (Fig. 3) and ITS gene (Supplementary Fig. S1) regions shows a close relationship with *P. pratensis* populations, but the subtle morphological differences, particularly in stylet length and tail shape, could reflect intraspecific variation or, alternatively, indicate that this population belongs to a yet undescribed species within the *Pratensis* group. Further investigations, including more extensive molecular and morphological analyses, would be necessary to clarify the taxonomic status of this population and

to determine whether it represents a new species within the *Pratylenchus* genus.

Prevalence of *Pratylenchus* species in New Zealand maize

The prevalence of *Pratylenchus* spp. across sampled maize fields in New Zealand revealed differences in prevalence (Fig. 5). *Pratylenchus neglectus* and *P. crenatus* were the most widely distributed species (Fig. 5A). Conversely, *P. thornei* and *P. pratensis* were found in the Canterbury region, while *P. penetrans* was detected only in the Waikato region. In this study, the prevalence of *Pratylenchus* species could be influenced by soil types and cropping history. *Pratylenchus thornei* was detected in three locations, characterized by loamy soils but different soil orders. In Lincoln, with Pallic (40%) and
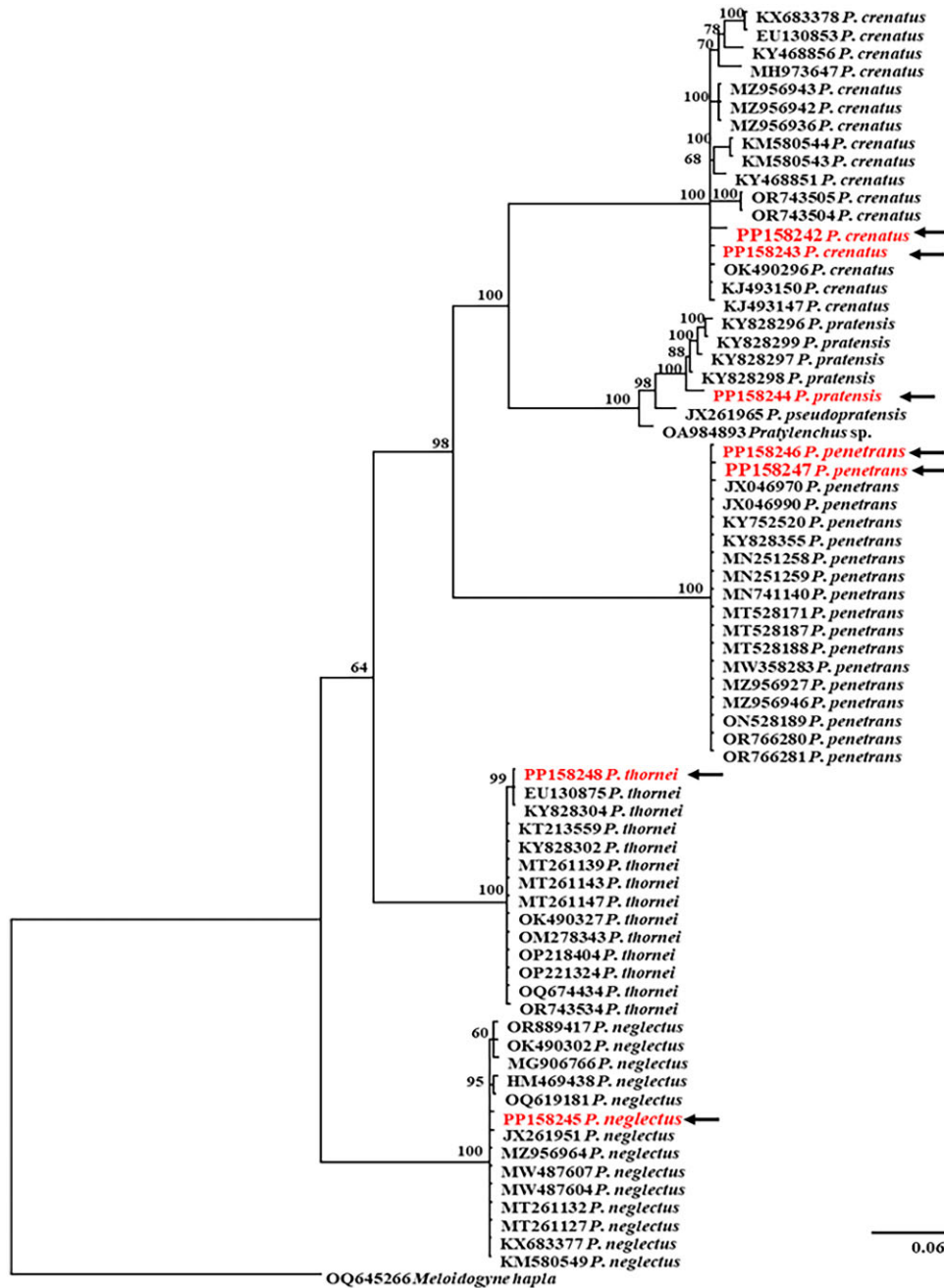
Figure 3. Bayesian tree inferred of *Pratylenchus* sequences LSU D2/D3 fragment of 28S rDNA under GTR + G + I model. The posterior probabilities of >50% are given in appropriate clades. Newly obtained sequences from New Zealand maize fields are indicated with arrows.

Recent (60%) soils, continuous maize cropping likely contributed to its persistence. In Darfield, where Pallic soils are dominant with stony loamy textures, *P. thornei* was found after maize cropping, followed by oat seedlings. In Lesston, where Pallic (75%) and Gley (25%) soils with loamy-clay textures are present, *P. thornei* thrived, particularly with crop rotations like maize and winter oats. In Dorie, *P. pratensis* was found in loamy Pallic soil, highlighting its adaptability to maize fields, especially after grass. Although less studied, its presence in loamy soils aligns with known patterns of nematode distribution. *Pratylenchus penetrans* was observed

in Cambridge, Matamata, and Otorohanga, areas with allophanic soils and continuous maize cropping, which likely supported its establishment. However, *P. crenatus* and *P. neglectus* prevailed in all fields that were sampled, except in Tahuna, where the organic soil order (peat soil) is present and no *Pratylenchus* was detected. The absence of root sampling in this study limits the understanding of *Pratylenchus* species in the root systems of maize. Future studies should include root data, alongside soil samples, for a more comprehensive understanding of their distribution. The co-occurrence of species and their abundance were not detailed but iden-
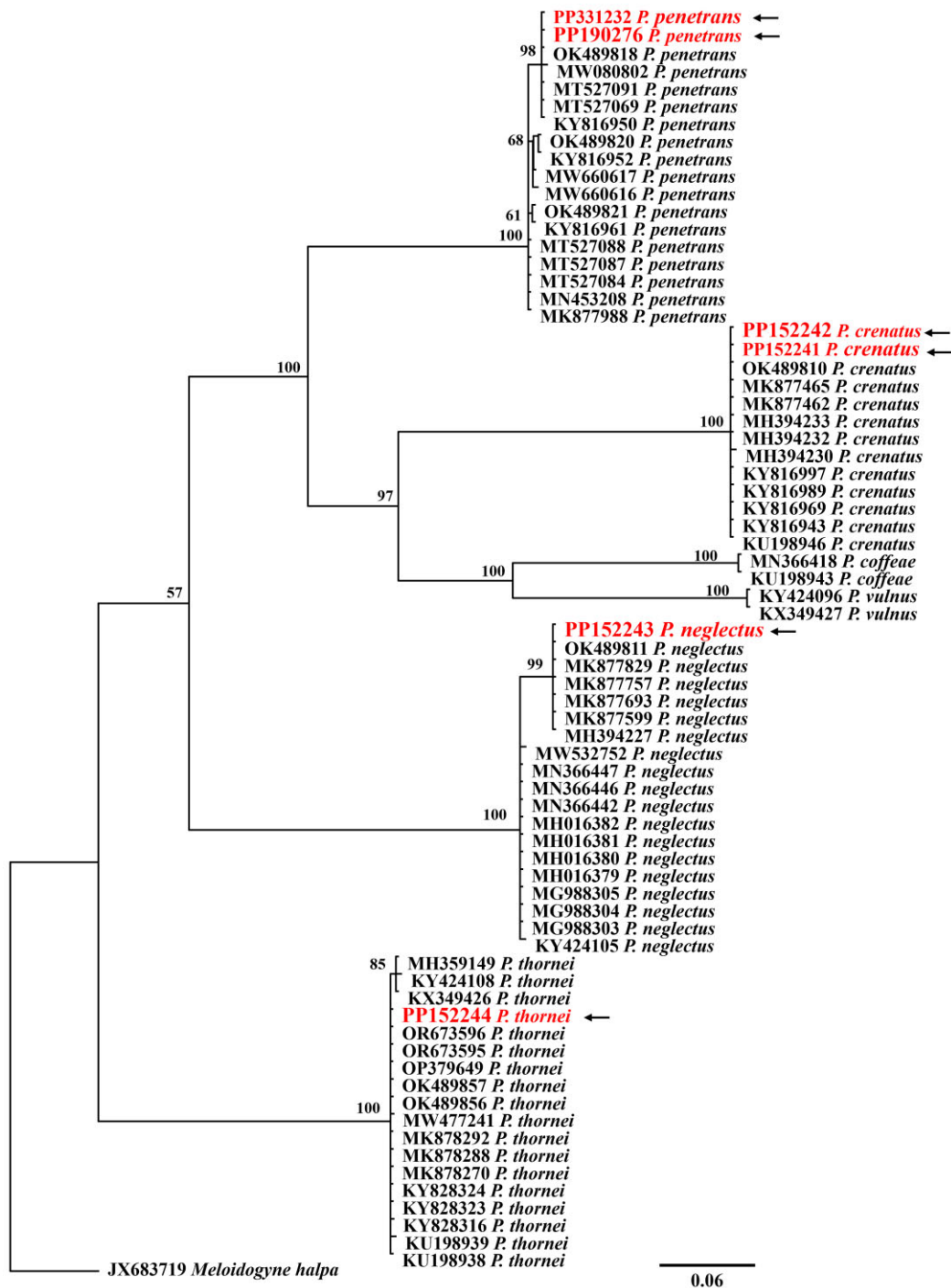
Figure 4. Bayesian tree inferred of *Pratylenchus* sequences COX1 of mtDNA under GTR + G model. The posterior probabilities of >50% are given in appropriate clades. Newly obtained sequences from New Zealand maize fields are indicated with arrows.

tified and confirmed through molecular and morphological analyses.

All these identified species are also reported in maize fields in different countries such as the USA, Australia, China, and the UK (Beane 1985, Thompson et al. 2008, Thompson et al. 2010, Simon et al. 2018, Xia et al. 2021, Simon et al. 2023, Thapa et al. 2023). Reported *Pratylenchus* species are polyphagous and can infect a range of other crops such as cereals, fruits, vegetables, forage crops, industrial crops, cot-

ton, coffee, potatoes, and ornamental plants, as well as weed species (Castillo and Vovlas 2007, Stirling 2023). In this study, *P. neglectus* and *P. crenatus* species were observed to be distributed within the New Zealand maize fields studied, and their abundance was also higher above 1000 kg^{-1} of soil in many locations.

Both nematode species, *P. neglectus* and *P. crenatus* were identified as potential pathogens and were associated with reduced growth in cereal crops, especially in maize and wheat
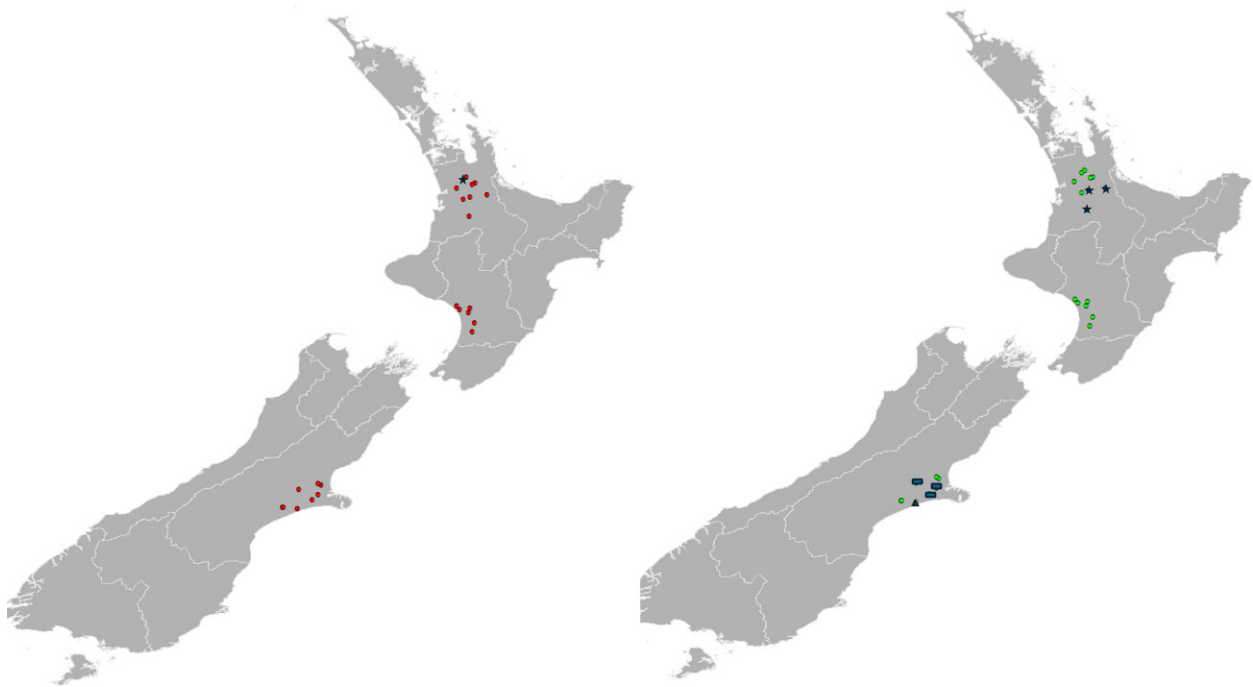
Figure 5. Prevalence of *Pratylenchus* species in sampled maize fields of New Zealand (A) circles indicate the *P. neglectus* and *P. crenatus* prevalence; (B) star indicate *P. penetrans*, rectamgles indicate *P. thornei*, and triangles indicate *P. pratensis*. In (A) star; (B) circles indicate the species that did not prevail.

(Castillo and Vovlas 2007, Simon et al. 2023). The New Zealand crop production sector is potentially at risk with PPN. Particularly *Pratylenchus* species reported in this study since they can infest multiple hosts (Stirling 2023). For instance, *P. penetrans* is associated with <400 different plant species (Ozbayrak et al. 2019). *Pratylenchus thornei* was reported in many countries as a significant pathogen to cereals, especially in wheat; for example, in Australia it is causing up to 85% yield losses in cereals (Knight 1996, Nicol et al. 1999). Additionally, *P. thornei* was reported in Canterbury wheat fields with a higher abundance (above 2000 kg^{-1} of soil) in New Zealand (Thiellier and Kularathna 2023), which is the threshold in Australian wheat (Thompson et al. 2010).

Conclusions

In conclusion, the findings of the study confirm that the *Pratylenchus* species associated with New Zealand maize are *P. neglectus*, *P. crenatus*, *P. thornei*, *P. penetrans*, and *P. pratensis*. The species distribution of *P. crenatus* and *P. neglectus* as the most prevalent species, along with localized distributions of *P. thornei*, *P. pratensis*, and *P. penetrans*, suggests potential regional variations in nematode populations, emphasizing the need for tailored management approaches based on local conditions.

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Author contributions

Nagarathnam Thiruchchelvan (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Investigation [lead], Methodology [lead], Software [lead], Validation [lead], Visualization [lead], Writing – original draft [lead], Writing – review & editing [supporting]), Manjula Kularathna (Conceptualization [lead], Formal analysis [supporting], Funding acquisition [lead], Investigation [supporting], Methodology [supporting], Project administration [lead], Resources [lead], Supervision [lead], Writing – review & editing [lead]), Romy Moukarzel (Conceptualization [supporting], Formal analysis [supporting], Methodology [supporting], Project administration [supporting], Resources [supporting], Supervision [supporting], Visualization [supporting], Writing – review & editing [lead]), Seona Casonato (Conceptualization [supporting], Formal analysis [supporting], Methodology [supporting], Supervision [supporting], Writing – review & editing [supporting]), and Leo M. Condron (Conceptualization [supporting], Supervision [supporting], Writing – review & editing [supporting])

Supplementary data

Supplementary data is available at *LAMBIO Journal* online.

Conflict of interest: Romy Moukarzel is on the editorial board of Letters in Applied Microbiology. She was not involved in the review or editorial process for this paper, on which she is listed as an author.

No conflict of interest was declared from other authors.

Data availability

Data can be obtained from the corresponding author.

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