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Plant-Parasitic Nematode Genera Associated with Turfgrass in Maryland Golf Courses and Athletic Fields

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Field surveys were conducted to assess the occurrence and diversity of plant-parasitic nematodes (PPNs) in golf courses and athletic fields across Maryland, USA, during 2022 and 2023. A total of 28 golf courses and ten athletic fields were surveyed, revealing the prevalence and abundance of 13 PPNs taxa in the region. *Criconemoides* was identified as the most prevalent (94.9%) and *Tylenchorhynchus* as the most abundant (2.3) across all samples. Central golf courses (west side of the Chesapeake Bay) exhibited a high prevalence of *Criconemoides* and *Tylenchorhynchus*, while Eastern Shore golf courses and athletic fields displayed a higher prevalence of *Helicotylenchus* and *Criconemoides*. Further, *Belonolaimus longicaudatus* was reported for the first time from turfgrass in Maryland, raising concerns due to its potential to cause severe damage on both cool- and warm-season turfgrass. Biodiversity analysis indicated that richness (R^2) was higher in athletic fields, while diversity (H') and evenness (J') were significantly greater in golf courses. This study provides baseline information for monitoring PPNs distribution in Maryland and also for the development of effective nematode management approaches in turfgrass ecosystems.

Keywords **:** distribution, diversity, plant parasitic nematodes, prevalence, turfgrass

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The turfgrass industry is a multibillion-dollar industry ($70-80 billion annually) that covers a vast amount of land, spanning over 62 million acres in the United States (Chawla et al., 2018). Turfgrass comprises various grass species that are grown and regularly maintained for use in parks, residential lawns, golf courses, and athletic fields. Sustainable turfgrass management benefits the environment by reducing runoff, preventing erosion, absorbing CO_2, and releasing O_2. Additionally, it enhances safety by acting as a barrier to fire damage, temperature regulation, and bioremediation (Reedich et al., 2017; Stier et al., 2013). Further, the turfgrass industry significantly boosts the U.S. economy through employment, spending on inputs, sales income, and services (Reedich et al., 2017). In 2002, it generated over $66 billion in revenue, where 44% belonged to golf courses (Haydu et al., 2008). Adjusted for inflation, this equals approximately $83 billion in 2020 (Stackhouse et al., 2020).

Turfgrass species in golf greens and athletic fields are susceptible to plant-parasitic nematodes (PPNs) damage (Dong et al., 2022; Vandenbossche et al., 2011). PPNs are microscopic roundworms that feed on plant roots. These organisms are ubiquitous in the soil environment. PPNs feed through a needle-like structure called a stylet that is used to puncture plant cells and ingest their contents (Hussey, 1989). A high abundance of PPNs can lead to root injury and limit the ability of the roots to uptake water and nutrients. In turfgrass, nematode damage leads to wilting, chlorosis, and reduction in turfgrass density (Crow and Han, 2005). Several PPNs genera including *Belonolaimus*, *Tylenchorhynchus*, *Helicotylenchus*, *Criconemoides*, *Hoplolaimus*, *Paratylenchus*, *Pratylenchus*, *Xiphinema*, *Meloidogyne*, and *Heterodera* have been repeatedly report-

ed from golf courses worldwide (Dong et al., 2022; Jordan and Mitkowski, 2006; Yu et al., 1998; Zeng et al., 2012). In Europe, turfgrass has been found to host over 50 different genera or taxa of PPNs (Vandenbossche et al., 2011). In the United States, 24 species from 19 different genera have been identified on multiple golf courses (Zeng et al., 2012).

While PPNs with varying feeding habits are associated with turfgrass, and it is common to find several species in one location, nematode damage might not be noticeable in the infested turfgrass. Visual symptoms are normally observed when the nematode population is high and the turfgrass is stressed (McLeod et al., 1994). Nematodes thrive in soils with high sand content. Turfgrass used for athletic fields and golf courses is often grown in sandy soil either through design in the case of the United States Golf Association (USGA) green construction or management practices such as frequent topdressing. The perennial presence of roots in golf greens can help nematodes survive throughout the year. In the southeast U.S., PPNs are some of the most economically important pathogens of warm-season golf green turfgrass. For example, *Belonolaimus longicaudatus* (sting) is known as the most damaging PPNs on bermudagrass in the southeast, with a damage threshold of less than 25 nematodes/100 cm^3 soil (Buckley et al., 2008; Crow, 2013).

PPNs can cause patchy yellowing and decline of turfgrass, which greatly diminishes the quality of the playing surface. When symptoms from PPNs feeding finally appear, roots are often seriously injured and may be slow to recover. Nematode feeding may also provide entry for other soilborne pathogens (Riedel, 1988). Chemical management is often used to reduce nematode counts, but identification of PPNs genera and quantification early in the growing season is important for timely management decisions. Therefore, timely nematode identification and quantification can be useful for anticipating potential problems and planning management actions such as proper cultural and chemical practices (Shao et al., 2023).

Turfgrass PPNs in northern U.S. regions are not as well studied as in southeastern turfgrass systems. It is unclear what impact PPNs have on northern golf greens and athletic fields. The shorter growing season and generally heavier native soil types (less favorable to PPNs) are currently thought to pose lower risks than in the southeast. However, as annual average temperatures become more unpredictable, turfgrass may experience more stress due to unfavorable temperatures. Stressed turfgrass could make golf greens more susceptible to injury from nematode feeding. Maryland is located in a climatic transition zone where both cool-season and warm-season turfgrass are grown. Since PPNs are not easily observed in a symptomatic area without soil extraction, PPNs may be incorrectly associated with symptoms when other biotic or abiotic factors have been ruled out, or not associated when actually contributing to symptoms. Documenting PPNs genera and relative abundance may provide useful information for future comparison. For example, turfgrass cultivars exhibit diverse levels of resistance/susceptibility to different nematode species (Jagdale et al., 2022; Pang et al., 2011a, 2011b). Little up-to-date information is available on nematodes associated with turfgrass in the northeast, especially on Maryland golf courses. The aim of this study was to determine I) the prevalence and distribution and II) the diversity of turfgrass PPNs in golf courses and some athletic fields in Maryland.

Materials and Methods

Sites and sample collection. Golf courses and athletic fields were sampled in Fredrick, Harford, Baltimore, Baltimore City, Anne Arundel, Montgomery, Prince George's, Talbot, Queen Anne's, and Worcester counties in Maryland (Fig. 1). Most samples were collected by the authors, a few samples were collected by golf course superintendents using the provided instructions. Golf courses and athletic fields were randomly selected; however, some locations with a history of nematode issues and symptoms such as thinning and yellowing were included. A total of 78 samples were collected in 2022 (n = 17) and 2023 (n = 61) from 28 golf courses (three courses were sampled in both years) and ten athletic fields. Three cool-season grasses, including creeping bentgrass (*Agrostis stolonifera*), annual bluegrass (*Poa annua*), and Kentucky bluegrass (KBG, *P. pratensis*) and two warm-season including zoysiagrass (*Zoysia* sp.) and bermudagrass (*Cynodon dactylon*) were represented (Table 1). The cultivar of each grass, if available, is specified in Table 1. A range of 15-20 soil cores with the size of 2 cm × 10 cm were collected per green or tee box and combined to form a single composite sample. In the athletic field, 20-25 cores were collected from each field with the exception of one baseball field, which was divided into two sections, and 15-20 cores were collected from each section. Samples were placed in a polyethylene bag and stored at 4°C prior to extraction. Samples were collected from June to July of 2022 and July to August of 2023.

Nematode extraction and identification. Nematodes were extracted from 100 cm^3 soil using sugar flotation and centrifugation (Jenkins, 1964). Briefly, the soil was placed on a sieve, which was placed on a 2-liter bucket. The soil

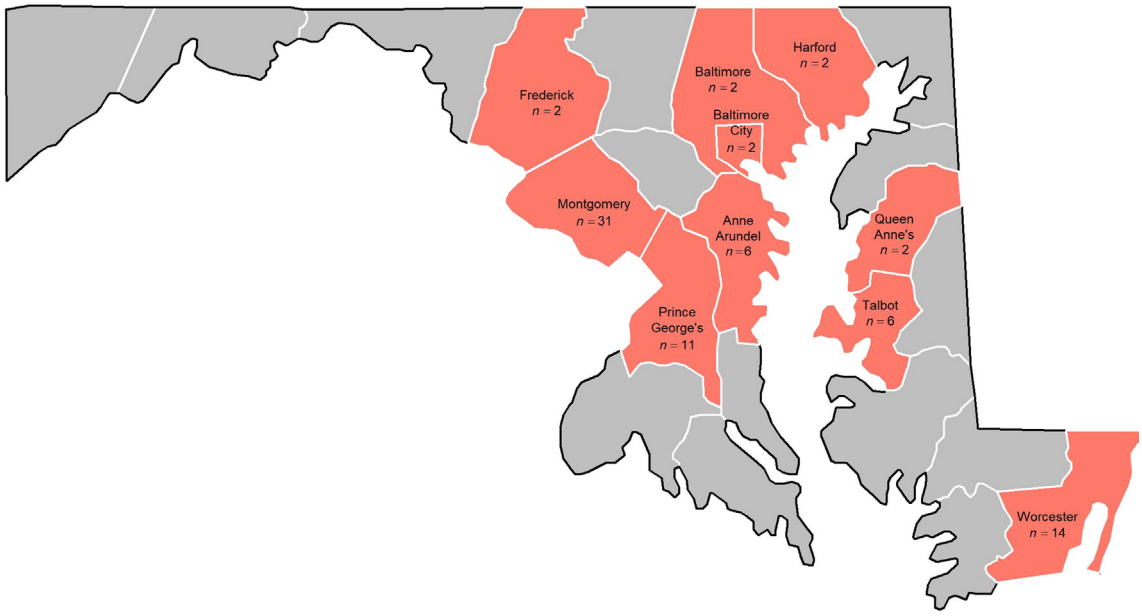

Fig. 1. Sampling sites and representative counties.

Table 1. Sampling site information

Site no.	Facility	Turfgrass	Establishment year	Sampling location	Soil type	Sampling year
1	South River Golf Club	Mixed cultivars_ creeping bentgrass (G), zoysiagrass (T)	1993	16, 3, 6 G, 2 T	Sandy	2022
2	Falls Road GC[a]	L93-Gran priix bentgrass	2003	3, 4, 5 G	Pushup green, sand on top	2022
3	Laytonsville GC[a]	Penncross bentgrass/poa	1992	3, 12, 15 G	Pushup green, sand on top 12 cm	2022
4	Andrews AFB GC[a]	Penncross creeping bentgrass/poa	1974	1, 2 South, 15 G	Sandy	2022
5	UMD Athletic Fields	Latitude + Tahoma bermudagrass	2019	Football Practice F	Sandy	2022
6	UMD Athletic Fields	Riviera bermudagrass	2006	Soccer Stadium F	Sandy	2022
7	UMD Athletic Fields	Mixture of bermudagrass cultivars	2003	Soccer Practice F	Sandy	2022
8	UMD Athletic Fields	Patriot + Latitude + Tahoma bermudagrass	2001	Softball Stadium F	Sandy	2022
9	Falls Road GC	L93-Gran priix bentgrass	2003	4, 14 G	Pushup green, sand on top 12 cm	2023
10	Poolesville GC	Pencross bentgrass/poa	1961	PG, 9 G	Pushup green, sand on top 12 cm	2023
11	Little Bennett GC	Penncross creeping bentgrass/poa	1999	6, 12 G	Sandy_California style	2023
12	Rattlewood GC	Penncross creeping bentgrass/poa	1962	4, 11 G	Sandy_ almost USGA style	2023
13	Laytonsville GC	Penncross bentgrass/poa	1992	1, 15 G	Pushup green, sand on top 12 cm	2023
14	Needwood GC	Penncross bentgrass/poa	1992	PG, 9 G	Sandy_ USGA style	2023
15	Northwest GC	Crenshaw creeping bentgrass/poa	1995	4, 6 G	Pushup green, sand on top 12 cm	2023
16	Hampshire Greens GC	Crenshaw creeping bentgrass/poa	1995	2, 12 G	Sandy_ USGA style	2023

(Continued)

Table 1. Continued

Site no.	Facility	Turfgrass	Establishment year	Sampling location	Soil type	Sampling year
17	Sligo Creek GC	Penncross creeping bentgrass/poa	1974	2, 3 G	Sandy_ USGA style	2023
18	Andrews AFB GC	Penncross creeping bentgrass/poa	1974	2, 17 G	Sandy	2023
19	The Cannon Club	Penncross creeping bentgrass/poa	1972	2 T, 15 G	Sandy	2023
20	TPC Potomac Golf Club	Penn A1-A4 creeping bentgrass	2008	15, 18 G	Sandy_ USGA style	2023
21	Congressional Country Club	Mixed cultivars_ creeping bentgrass/poa	2020 (4), 1994 (17)	4, 17 G	Sandy (4), push up (17)	2023
22	Cabin John Regional Athletic Area	NorthBridge and Latitude 96 bermudagrass	-[b]	1 F	Native soil (sand infield)	2023
23	Cabin John Regional Athletic Area	Tall fescue	-[b]	4 F	Native soil	2023
24	Cabin John Regional Athletic Area	NorthBridge and Latitude 96 bermudagrass	-[b]	6 F	Native soil	2023
25	Oriole Park at Camden Yards	MVS 365SS Kentucky bluegrass	2022	West, East F	Sandy	2023
26	Under Armour Performance Center	NorthBridge bermudagrass	2021 and 2023	2 F	Root zone mix-sand	2023
27	Under Armour Performance Center	NorthBridge bermudagrass	2021	3 F	Root zone mix-sand	2023
28	Cove Creek Club	Creeping bentgrass/poa	1980	4, 8 G	Sandy	2023
29	Hog Neck GC	L-93 creeping bentgrass/poa annua	1998	1, 16 G	Sandy	2023
30	Talbot Country Club	Penncross creeping bentgrass/poa	1999 (5), 1960 (4)	5, 14 G	Sandy_ USGA style (5), sand modified push up (14)	2023
31	Links at Perry Cabin	A1-A4 and 007 creeping bentgrass	2018 (9), 2019 (13)	9, 13 G	Sandy	2023
32	Glen Riddle GC (Man O Loar)	Creeping bentgrass	2005-2006	6, 15 G	Green mix (80 sand/20 peat)	2023
33	Glen Riddle GC (War Admirac)	Creeping bentgrass	2005-2006	10, 18 G	Green mix (80 sand/20 peat)	2023
34	River Run Golf and Country Club	Penncross creeping bentgrass/poa (14), Pennlinks creeping bentgrass/poa (16)	1991	14, 16 G	Sandy_ USGA style	2023
35	Ocean City Golf Club	Mixed cultivars_ creeping bentgrass	1960 (17), 1998 (Newport)	17 G, Newport Bay G	Native soil, sandy_ USGA style	2023
36	Eagle's Landing GC	Penncross, 007 creeping bentgrass	1990	6, 9 G	Sandy	2023
37	Rum Pointe Seaside Golf Links	Penncross creeping bentgrass/poa	1997	10 G, PG	Sandy	2023
38	The Links at Lighthouse Sounds GC	Creeping bentgrass	1999	15 T, 9 G	Native soil, Sandy_ USGA style	2023
39	Maryland National Golf Club	L93 creeping bentgrass	2002	6, 16 G	Sandy	2023
40	Mountain Branch Golf Club	L93 creeping bentgrass	2000	17, 18 G	Sandy_ USGA style	2023
41	Woodmore Country Club	Creeping bentgrass/poa	1980	15, 16 G	Push up native with sand on top	2023

F, field; GC, golf course; G, putting green; PG, practice green; T, tee.
[a]The golf course was sampled in 2022 and 2023; however, sampling locations were different.
[b]Information is not available.

was washed with tap water into the bucket. Material captured on a 25-µm sieve was centrifuged in 45.4% sucrose solution at 3000 RPM for 3 min to separate nematodes. The suspension was poured over a 25-µm sieve and thoroughly rinsed with tap water, and then collected into a 50 ml tube for identification and counting. Nematodes were identified to the genus level based on morphological features using an inverted microscope (Zeiss, Oberkochen, Germany) and quantified. The data were recorded as the number of nematodes in 100 cm^3 of soil. The key characteristics used for identification were the head, stylet, lip area, medium bulb, digestive system, tail, and reproductive system (Van den Berg et al., 2017). Nematodes were identified to genus level using a pictorial key by Mai et al. (1996). Further, upon morphological identification of *Belonolaimus* sp., which is the first report of the genus from Maryland, the internal transcribed spacer region internal transcribed spacer (ITS) 1 and 2 and 28S large ribosomal subunit D2-D3 expansion region were sequenced to determine the species.

Data analysis. All analyses were conducted in SAS (version 9.4, SAS Institute, Cary, NC, USA). Levene's test for homogeneity of variances ($P \leq 0.05$) was conducted to determine whether data from both years could be combined for analysis. The prevalence and abundance of PPNs taxa were determined as described by Sawadogo et al. (2009). Prevalence was calculated by dividing the number of samples containing a specific nematode by the total number of samples, then converted to a percentage. To calculate the prevalence for each turfgrass species, the total number of samples in that turfgrass species was used. Abundance was measured using a logarithmic scale based on average nematodes in 100 cm^3 of soil. This approach aids in reducing skewness, handling zero counts, and effectively scaling the data.

Prevalence: $e/N \times 100$

Abundance: $\log10[(N_i/P) + 1]$

Where e = the number of samples with the specific taxa and N = the total number of samples. N_i = the number of a specific nematode across all samples and P = the total number of positive samples for that nematode.

There is no threshold level for nematode damage in turfgrass in Maryland; however, there is one established for the U.S. Northeast (New England) golf greens (https://ag.umass.edu/turf/fact-sheets/assessing-role-of-nematodes-in-putting-green-decline) which we used to determine if specific taxa are potentially in a damaging range. The nematode count (number of individuals per 100 cm^3 of soil) was used for comparison.

Additionally, the following diversity indices, including richness (R^2), diversity (H'), and evenness (J'), were calculated for central golf courses (golf courses on the west side of the Chesapeake Bay), Eastern Shore golf courses (golf courses on the east side of the Chesapeake Bay), and athletic fields (Menhinick, 1964; Yeates and Bird, 1994).

Richness (R^2) = $S/\sqrt{N}$

Shannon Diversity Index (H') = $-\sum(pi \times \ln(pi))$

Evenness (J') = $H'/\ln(S)$

Where S = the number of taxa, N = the total number of samples for each region/turfgrass setting, and pi = proportion of individuals of taxon i in the total population.

The differences in diversity indices were examined using Duncan's multiple range test, with significance set at $P < 0.05$.

Results

Prevalence and abundance. Levene's test for homogeneity of variances ($P \leq 0.05$) was not significant, and therefore, a combined analysis across years was conducted. All soil samples contained PPNs, and a total of 13 taxa were identified from all golf courses and athletic fields sampled in 2022 and 2023. Supplementary Table 1 indicates the average counts of each taxon per site. The most frequently observed taxon was *Criconemoides* (ring), with a prevalence of 94.9%, which was present in 74 samples, followed by *Tylenchorhynchus* (stunt) and *Helicotylenchus* (spiral) detected in 70 and 69 samples (prevalence of 89.7% and 88.5%), respectively. The least frequent nematodes were *Belonolaimus* (sting), *Longidorus* (needle), and *Paratylenchus* (Pin), with a prevalence of 1.3%, where each was identified only in one sample. All other nematodes had a prevalence range between 3.8% to 82.1% (Table 2). In terms of abundance, *Tylenchorhynchus*, with an abundance of 2.3, was ranked first, followed by *Criconemoides, Helicotylenchus*, and *Hoplolaimus*, with an abundance of 2.1. *Longidorus* had the lowest abundance (0.3) compared to all other taxa. All other taxa had an abundance ranging between 0.8-1.6.

The prevalence and abundance were also calculated separately for central golf courses, Eastern Shore golf courses, and athletic fields. In central golf courses (n = 45), the prevalence of *Criconemoides* and *Tylenchorhynchus* (95.6%) was the highest, followed by *Helicotylenchus* and *Hoplolaimus*, with a prevalence of 82.2%. *Tylenchorhynchus* and *Criconemoides* had the highest abundance (2.3 and 2.0, respectively). In Eastern Shore golf courses (n = 22), *Helicotylenchus* and *Criconemoides*, with a prevalence

Table 2. Nematode prevalence, abundance, and samples above the damage threshold in golf courses and athletic fields in Maryland

Nematode taxon	% Prevalence (n = 78)[a]	Abundance	New England threshold/100 cm^3 [b]	Highest count/100 cm^3	% Samples above the threshold
Belonolaimus	1.3	0.7	-[c]	4	-
Criconemoides	94.9	2.1	1,500	1,010	0
Helicotylenchus	88.5	2.1	1,500	811	0
Hemicycliophora	5.1	1.6	200	82	0
Heterodera	10.3	1.2	500	55	0
Hoplolaimus	82.1	2.1	400	1012	5.1
Longidorus	1.3	0.3	100	1	0
Meloidogyne	62.8	1.1	500	103	0
Paratylenchus	1.3	1.6	-	42	-
Pratylenchus	32.1	1.5	100	157	3.8
Trichodoridae[d]	82.1	1.4	-	145	-
Tylenchorhynchus	89.7	2.3	800	1,284	2.6
Xiphinema	3.8	0.8	-	14	-

[a]n = total number of samples.
[b]The document can be reached at: https://ag.umass.edu/turf/fact-sheets/nematodes-on-golf-greens.
[c]The threshold is not determined.
[d]Includes *Trichodorus* and *Paratrichodorus* genera.

of 95.5% and an abundance of 2.4 and 2.2, respectively, were ranked on top followed by Trichodoridae (*Trichodorus* and *Paratrichodorus*, stubby root) with a prevalence of 81.8% and abundance of 1.6. In athletic fields (n = 11), *Helicotylenchus* and *Criconemoides* had the highest prevalence compared to other taxa, 14.1% and 12.8%, respectively, with Meloidogyne ranked in the second position with 9% prevalence. The abundance of *Hemicycliophora* (sheath), *Criconemoides*, and *Helicotylenchus* (1.8, 1.8, and 1.7, respectively) was the highest in samples collected from athletic fields. This indicates a slight difference among the prevalence and abundance of nematode taxa in different turfgrass settings and geographical locations (central vs. Eastern Shore); however, *Criconemoides* and *Helicotylenchus* were frequently found in most samples regardless of the location and turfgrass setting (Table 2).

First report of *Belonolaimus*. *Belonolaimus* was identified only in one athletic field, with North Bridge bermudagrass established in 2021 and partially sodded in 2023. The density was four in 100 cm^3 of soil, and no noticeable symptoms were associated with nematode injuries. Morphological characteristics and sequencing of ITS and 28S large ribosomal regions resulted in the identification of *B. longicaudatus* (GenBank accession no. OR520202 and OR520203 for ITS and OR520268 and OR520269 for 28S) in turfgrass for the first time in Maryland (Waldo et al., unpublished). Continued assessments will be essential to monitor and ascertain whether the population of *Belonolaimus* in Maryland reaches a critical threshold that could potentially cause damage.

Counts and damage thresholds. Nematode counts of each taxon were compared with a previously established nematode damage threshold in turfgrass for the Northeast region. *Hoplolaimus*, with a maximum population size of 1,012 nematodes in 100 cm^3 of soil, had higher counts than the threshold in 5.1% of the samples. *Pratylenchus* and *Tylenchorhynchus* populations were also above the threshold in 3.8% and 2.6% of the samples (Table 2). Some golf courses with nematode counts higher than the New England threshold showed symptoms such as general turfgrass weakness and thinning during summer, especially during the heat stress period.

Prevalence by turf species. The prevalence of the nematode taxa in golf courses and athletic fields was also calculated based on turfgrass species. The dominant turfgrass species in golf courses was creeping bentgrass and annual bluegrass mixture (n = 50), while 16 samples were creeping bentgrass, and one sample was zoysiagrass. The majority of sampled athletic fields had bermudagrass (n = 8), while two samples were KBG, and one sample was tall fescue (Tables 1 and 3). In golf courses, *Criconemoides*

Table 3. Plant-parasitic nematode prevalence from Maryland based on turfgrass species

Nematode taxon	% Prevalence					
	Golf courses			Athletic fields		
	Creeping bentgrass (n = 16)	C. bentgrass + annual bluegrass (n = 50)	Zoysia (n = 1)	Bermudagrass (n = 8)	Kentucky bluegrass (n = 2)	Tall fescue (n = 1)[a]
Belonolaimus	0	0	0	12.5	0	0
Criconemoides	100	96.0	0	100	100	0
Helicotylenchus	93.8	84.0	100	100	100	100
Hemicycliophora	6.3	2.0	0	0	100	0
Heterodera	18.8	6.0	0	12.5	50.0	0
Hoplolaimus	93.8	88.0	0	50.5	0	100
Longidorus	0	2.0	0	0	0	0
Meloidogyne	50.0	66.0	100	75.0	0	100
Paratylenchus	0	0	0	0	0	100
Pratylenchus	18.8	36.0	0	25.0	50.0	100
Trichodoridae[b]	81.3	86.0	0	87.5	50.0	0
Tylenchorhynchus	93.8	100	0	25.0	100	100
Xiphinema	0	0	0	25.0	0	100

[a]Present taxa have a prevalence of 100% due to the sample size of one.
[b]Includes *Trichodorus* and *Paratrichodorus* genera.

was detected in 100% of the creeping bentgrass samples, while *Helicotylenchus*, *Hoplolaimus*, and *Tylenchorhynchus* were isolated from 93.8% of the samples. On creeping bentgrass and annual bluegrass mixture, *Tylenchorhynchus* had 100% prevalence, followed by *Criconemoides* with 96% prevalence. The only two taxa identified in zoysiagrass samples were *Helicotylenchus* and *Meloidogyne*. In athletic fields' bermudagrass samples, *Criconemoides* and *Helicotylenchus* had 100% prevalence. In KBG samples, the prevalence of four species, including *Criconemoides*, *Helicotylenchus*, *Hemicycliophora*, and *Tylenchorhynchus* was 100. Seven taxa were present in the tall fescue sample (Table 3).

Biodiversity. Three biodiversity indices, including R^2, H', and J' showed significant differences among golf course and athletic field samples. R^2 was significantly higher for PPNs in athletic field samples (0.24) than golf courses. There were no significant differences in R^2 among the golf courses on the two sides of the Chesapeake Bay. J' showed significant differences among golf courses and athletic field samples, where it was significantly higher in Eastern Shore golf courses (0.75), followed by central golf courses (0.66) and lastly in athletic fields (0.56). H' was significantly higher in golf courses (1.53 and 1.55) compared to athletic fields (1.44) (Table 4).

Discussion

The present study was conducted to evaluate the occurrence, prevalence, and diversity of PPNs in golf courses and athletic fields in Maryland. We examined PPNs in

Table 4. Diversity of plant-parasitic nematodes in Central and Eastern Shore golf courses and athletic fields in Maryland

Site	No. of samples	Richness (R^2)[a]	Evenness (J')	Diversity (H')
Central golf courses	45	0.07 b	0.66 b	1.53 a
Eastern Shore golf courses	22	0.06 b	0.75 a	1.55 a
Athletic fields	11	0.24 a	0.56 c	1.44 b

[a]Means followed by the same letters in a column are not significantly different at $P < 0.05$ by Duncan's multiple range test.

38 golf courses and athletic fields, revealing their widespread presence in the region. We identified 13 nematode taxa, with *Criconemoides* being the most prevalent and *Tylenchorhynchus* the most abundant across all samples. *Criconemoides* and *Tylenchorhynchus* were the most frequent taxa in central golf courses, while *Helicotylenchus* and *Criconemoides* had the highest prevalence in Eastern Shore golf courses and athletic fields. *Belonolaimus* was identified in an athletic field, marking its first report from Maryland. Some sites exceeded established nematode damage thresholds, leading to some issues in general turfgrass health and quality. Biodiversity analysis showed variations among locations and turfgrass settings.

Of the 13 taxa identified in this study, six taxa including *Heterodera*, *Longidorus*, *Xiphinema*, *Hemicycliophora*, *Belonolaimus*, and *Paratylenchus* were detected in less than eight samples (10% of the total samples). For example, *Xiphinema* was detected only in athletic fields and *Hemicycliophora* was not detected in central golf courses. The regional distribution of PPNs has been reported previously (Bond et al., 2000; Dong et al., 2022; Liu et al., 2009; Simard et al., 2008; Yingjun, 2006). While Maryland is a relatively small state in terms of land area, it has diverse geographical features contributing to a comparatively wide range of climatic conditions which can explain this distribution.

According to Dong et al. (2022), an abundance greater than 1 can potentially cause damage to turfgrass. While the majority of detected nematode taxa had abundances above 1, the absence of observed damage could be attributed to a combination of factors, including environmental conditions, management practices, and threshold variability.

PPNs densities in some samples exceeded the New England turfgrass nematode threshold; however, we observed actual turfgrass damage in only a limited number of sites. Interestingly, certain golf courses that had not shown symptoms of nematode-related issues in recent years reported problems in the past. These courses have since implemented nematicide programs or adjusted their cultural practices such as aerification, application of wetting agents, and irrigation patterns. These proactive measures are believed to have played a pivotal role in enhancing turfgrass health (communication with golf course superintendents through a survey associated with the sampling). This observation underscores the significance of effective management practices, as they can help mitigate the stress on turfgrass, even when PPNs levels are elevated. Further research is warranted to establish nematode threshold levels tailored to Maryland's weather conditions, management practices, and other factors that may differ from those of New England. Such region-specific thresholds will provide valuable guidance for turfgrass managers in Maryland.

In this study, no major differences in the number of PPNs among the turfgrass species were observed. Mwamula and Lee (2021) indicated species specificity being restricted to a few uncommon PPNs in turfgrass such as *Paratylenchus nanus* with low prevalence and high intensity, relating this finding to extreme host specificity of uncommon PPNs. Zeng et al. (2012) reported a higher number of PPNs in bermudagrass than in creeping bentgrass; however, they identified the soil type as a more determinant factor in PPNs prevalence compared to turfgrass species. Crow (2005) reported a higher damage capability by PPNs in sandy soils. In our study, 21 samples (27%) had original native soil (or push-up greens), and the rest were sandy soil. Among the samples with native soil, only three had nematode densities above the damage threshold. This observation can likely be attributed to a common practice of topdressing with sand on golf courses. As a consequence, the uppermost layers of soil tend to consist predominantly of sand.

Species biodiversity indices, including J', R^2, and H', showed significant differences among golf courses and athletic fields. J' and H' were lower in athletic fields, indicating a lower diversity of taxa compared to golf courses. This variation can be explained by the differences in the level of turfgrass management intensity between athletic fields and golf courses. Athletic fields tend to employ less rigorous turfgrass maintenance practices compared to golf courses. In contrast, the intensive management practices often applied on golf courses may subject turfgrass to higher stress levels, potentially creating favorable conditions for survival and increased diversity of PPNs. R^2 is affected by sample size (Plog and Hegmon, 1993); therefore, the higher R^2 of athletic fields is more likely due to a smaller sample size in this study. The only diversity index that was significantly different between central and Eastern Shore golf courses was J'. Higher evenness in PPNs from Eastern Shore samples could be attributed to sandy soil being the dominant soil type and differences in the climate on the two sides of the Chesapeake Bay. Other studies have also shown the role of soil texture and climate on diversity indices of PPNs in turfgrass (Yeates and Bongers, 1999; Zeng et al., 2012).

While we found *Belonolaimus* in only one of the athletic fields with a population of four nematodes per 100 cm^3 of soil, this could be concerning. *Belonolaimus* has the potential to cause damage with 20 to 25 nematodes per 100 cm^3 of soil, and it is considered a major turfgrass PPN in the southeast (Crow, 2013). Considering the ongoing climate change and shifts in temperature patterns, regular monitoring of the density and distribution of this nematode taxon

in Maryland is essential to predict potential issues in the future. Additionally, with the constant increase in the use of bermudagrass in Maryland due to its adaptability and resilience in the transition zone, it is important to continue monitoring *Belonolaimus* population density and distribution in the state.

This survey was conducted during the summers of 2022 and 2023 and offered valuable insights into nematode prevalence and distribution in Maryland. However, additional monitoring and sampling across different seasons are advisable to gain a more comprehensive understanding. Late spring to mid-summer is recognized as the peak season for certain PPN taxa, such as *Tylenchorhynchus*, while others, such as *Helicotylenchus*, *Criconemoides*, *Hoplolaimus*, and *Heterodera* have been documented to have elevated population densities during the fall (Jordan and Mitkowski, 2006; Settle et al., 2006). Conducting sampling in both spring and fall in Maryland could yield further information about the prevalent and abundant taxa identified in this study, including *Criconemoides*, *Tylenchorhynchus*, and *Helicotylenchus*. Similarly, the density of *B. longicaudatus* on bermudagrass has been reported to fluctuate in different seasons (McGroary et al., 2009). Although this species had a low prevalence in this study, monitoring its population dynamics is crucial.

This research provides information about the occurrence, geographical distribution, and diversity of PPNs in golf courses and athletic fields in Maryland. A total of 13 taxa were reported, where *Criconemoides*, *Tylenchorhynchus*, *Helicotylenchus*, and *Hoplolaimus* were the most prevalent and abundant across all samples. Further, *Belonolaimus* was reported for the first time on turfgrass in the state. While certain golf courses exhibited PPNs densities surpassing established Northeast thresholds, not all of them displayed symptoms associated with nematode damage. The diversity indices were significantly different among turfgrass settings, indicating varying potential risks in golf courses versus athletic fields. These findings emphasize the need for consistent monitoring and the establishment of nematode threshold levels tailored to the state to effectively manage PPNs in turfgrass.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

References

Bond, J. P., McGawley, E. C. and Hoy, J. W. 2000. Distribution of plant-parasitic nematodes on sugarcane in Louisiana and efficacy of nematicides. *J. Nematol.* 32:493-501.

Buckley, R. J., Koppenhöfer, A. M. and Crow, W. T. 2008. Integrated approach to nematode management in turfgrass. *Plant Pest Advis*. 14:4-7.

Chawla, S. L., Agnihotri, R., Patel, M. A., Patil, S. and Shah, H. P. 2018. Turfgrass: a billion dollar industry. In: *National Conference on Floriculture for Rural and Urban Prosperity in the Scenario of Climate Change-2018*, pp. 30-35. Indian Council of Agricultural Research, National Research Centre for Orchids, Pakyong, India.

Crow, W. T. 2005. Plant-parasitic nematodes on golf course turf. *Outlooks Pest Manag.* 16:10-15.

Crow, W. T. 2013. Nematode management for golf courses in Florida. University of Florida, IFAS Extension. ENY-008/IN124. Institute of Food and Agricultural Sciences (IFAS), Gainesville, FL, USA. 8 pp.

Crow, W. T. and Han, H. 2005. Sting nematode. *Plant Health Instr.* Online publication. https://doi.org/10.1094/PHI-I-2005-1208-01.

Dong, Y., Jin, P., Zhang, H., Hu, J., Lamour, K. and Yang, Z. 2022. Distribution and prevalence of plant-parasitic nematodes of turfgrass at golf courses in China. *Biology* 11:1322.

Haydu, J. J., Hodges, A. W. and Hall, C. R. 2008. Estimating the economic impact of the U.S. golf course industry: challenges and solutions. *HortScience* 43:759-763.

Hussey, R. S. 1989. Disease-inducing secretions of plant-parasitic nematodes. *Annu. Rev. Phytopathol*. 27:123-141.

Jagdale, G. B., Nugraha, G. T., Martin, K., Martinez-Espinoza, A. D. and Hajihassani, A. 2022. Occurrence of the lance nematode *Hoplolaimus stephanus* infecting bentgrass *Agrostis stolonifera* in Georgia, U.S.A. *Plant Health Prog*. 23:162-165.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis. Rep*. 48:692.

Jordan, K. S. and Mitkowski, N. A. 2006. Population dynamics of

plant-parasitic nematodes in golf course greens turf in Southern New England. *Plant Dis*. 90:501-505.

Liu, Q.-Z., Liang, L.-L., Yang, D., Bian, Y., Zhou, H.-Y. and Xie, D.-Y. 2009. Analysis of community characteristics of nematodes in turf soil of Yongfeng virescence region in Beijing. *Acta Pratacult. Sin*. 18:136-141.

Mai, W. F., Mullin, P. G., Lyon, H. H. and Loeffler, K. 1996. Plant-parasitic nematodes: a pictorial key to genera. Cornell University Press, Ithaca, NY, USA. 277 pp.

McGroary, P., Crow, W. T., McSorley, R., Giblin-Davis, R. M. and Cisar, J. L. 2009. Seasonal fluctuations of *Belonolaimus longicadatus* in bermudagrass. *Nematropica* 39:99-110.

McLeod, R., Reay, F. and Smyth, J. 1994. Plant nematodes of Australia listed by plant and by genus. NSW Agriculture, Rydalmere, New South Wales, Australia. 201 pp.

Menhinick, E. F. 1964. A comparison of some species-individuals diversity indices applied to samples of field insects. *Ecology* 45:859-861.

Mwamula, A. O. and Lee, D. W. 2021. Occurrence of plant-parasitic nematodes of turfgrass in Korea. *Plant Pathol. J*. 37:446-454.

Pang, W., Luc, J. E., Crow, W. T., Kenworthy, K. E., Giblin-Davis, R. M., McSorley, R. and Kruse, J. K. 2011a. Bermudagrass cultivar responses to sting nematodes. *Crop Sci*. 51:2199-2203.

Pang, W., Luc, J. E., Crow, W. T., Kenworthy, K. E., McSorley, R., Kruse, J. K. and Giblin-Davis, R. M. 2011b. Responses of seashore paspalum cultivars to sting and spiral nematodes. *Crop Sci*. 51:2864-2867.

Plog, S. and Hegmon, M. 1993. The sample size-richness relation: the relevance of research questions, sampling strategies, and behavioral variation. *Am. Antiq.* 58:489-496.

Reedich, L. M., Millican, M. D. and Koch, P. L. 2017. Temperature impacts on soil microbial communities and potential implications for the biodegradation of turfgrass pesticides. *J. Environ. Qual*. 46:490-497.

Riedel, R. M. 1988. Interactions of plant-parasitic nematodes with soil-borne plant pathogens. *Agric. Ecosyst. Environ*. 24:281-292.

Sawadogo, A., Thio, B., Kiemde, S., Drabo, I., Dabire, C., Ouedraogo, J., Mullens, T. R., Ehlers, J. D. and Roberts, P. A. 2009. Distribution and prevalence of parasitic nematodes of cowpea (*Vigna unguiculata*) in Burkina Faso. *J. Nematol*. 41:120-127.

Settle, D. M., Fry, J. D., Todd, T. C. and Tisserat, N. A. 2006. Population dynamics of the lance nematode (*Hoplolaimus galeatus*) in creeping bentgrass. *Plant Dis*. 90:44-50.

Shao, H., Zhang, P., Peng, D., Huang, W., Kong, L.-A., Li, C., Liu, E. and Peng, H. 2023. Current advances in the identification of plant nematode diseases: from lab assays to in-field diagnostics. *Front. Plant Sci*. 14:1106784.

Simard, L., Bélair, G., Powers, T., Tremblay, N. and Dionne, J. 2008. Incidence and population density of plant-parasitic nematodes on golf courses in Ontario and Québec, Canada. *J. Nematol.* 40:241-251.

Stackhouse, T., Martinez-Espinoza, A. D. and Ali, M. E. 2020. Turfgrass disease diagnosis: past, present, and future. *Plants* 9:1544.

Stier, J. C., Steinke, K., Ervin, E. H., Higginson, F. R. and McMaugh, P. E. 2013. Turfgrass benefits and issues. In: *Turfgrass: biology, use, and management*, eds. by J. C. Stier, B. P. Horgan and S. A. Bonos, Vol. 56, pp. 105-145. American Society of Agronomy, Soil Science Society of America, Crop Science Society of America, Madison, WI, USA.

Van den Berg, E., Marais, M. and Swart, A. 2017. Nematode morphology and classification. In: *Nematology in South Africa: a view from the 21st century*, eds. by H. Fourie, V. W. Spaull, R. K. Jones, M. S. Daneel and D. De Waele, pp. 33-71. Springer, Cham, Switzerland.

Vandenbossche, B., Viaene, N., Sutter, N., Maes, M., Karssen, G. and Bert, W. 2011. Diversity and incidence of plant-parasitic nematodes in Belgian turf grass. *Nematology* 13:245-256.

Yeates, G. W. and Bird, A. F. 1994. Some observations on the influence of agricultural practices on the nematode faunae of some South Australian soils. *Fundam. Appl. Nematol*. 17:133-145.

Yeates, G. W. and Bongers, T. 1999. Nematode diversity in agroecosystems. *Agric. Ecosyst. Environ*. 74:113-135.

Yingjun, W. 2006. Investigation and identification of species of parasitic nematodes in grass in Huaian region. *J. Anhui Agric. Sci*. 34:3413.

Yu, Q., Potter, J. W. and Gilby, G. 1998. Plant-parasitic nematodes associated with turfgrass in golf courses in southern Ontario. *Can. J. Plant Pathol.* 20:304-307.

Zeng, Y., Ye, W., Martin, S. B., Martin, M. and Tredway, L. 2012. Diversity and occurrence of plant-parasitic nematodes associated with golf course turfgrasses in North and South Carolina, USA. *J. Nematol*. 44:337-347.