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A First Report of *Anguina pacificae* in Ireland

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Abstract: Anguina pacificae is a significant pest of *Poa annua* golf course greens in northern California. This study presents the first confirmed case of an *A. pacificae* infestation outside of North America, where the nematode's distribution is further restricted to a relatively limited coastal region. Species confirmation was made by morphometric and molecular methods and comparisons to closely related species including the European species, *Anguina agropyri*. The *A. pacificae* population detected on an Irish golf course was monitored over a 2-yr period and the life cycle compared with Californian population dynamics. *A. pacificae* was assessed for the potential risk of spreading to the local agricultural sector, in addition, the biosecurity risks from *A. pacificae* and plant parasitic nematodes in general were reviewed for northwest Europe.

Key words: Agrostis, *Anguina pacificae*, annual bluegrass, biosecurity, detection, diagnosis, host range, morphology, morphometrics, Pacific shoot-gall nematode, *Poa annua*, population dynamics, stem gall.

Since the 18th century, nematodes of the genus *Anguina* have been recorded parasitizing grasses and cereals across Europe. Although most plant parasitic nematodes attack the root systems of their hosts, *Anguina* typically attack the leaves, flowers, seeds, and stems of plants. The type species for the genus, the wheat seed-gall nematode *Anguina tritici*, induces seed galls and can be a serious pest on wheat and barley crops (Goodey and Hooper, 1958) with yield losses of 32% reported for infected durum wheat (Ozberk et al., 2011). In Russia and other parts of Europe, fodder grasses and seed yields can be severely affected by another species, *Anguina agrostis* (Chizhov, 1980). Modern seed cleaning techniques have eliminated many of the problems caused by *Anguina* species in European agriculture but *Anguina agropyri*, the couch-grass nematode, continues to cause significant damage, inducing stem galls in wheat, rye, and barley within Estonia, Latvia, Lithuania, and Russia. This species is particularly aggressive, with as few as 2 to 5 *A. agropyri* juveniles causing stunting and serious yield losses in cereals (Krall, 1991). An additional problem caused by *A. tritici* and related species such as *Anguina funesta* is the transmission of the bacterial pathogen *Rathayibacter toxicus* that produces toxins in forage grasses often resulting in the fatal poisoning of grazing animals (Riley, 1992, 1995). Similarly, other *Rathayibacter* spp. have subsequently been identified in *Anguina graminis* leaf galls (Dorofeeva et al., 2002).

In Ireland, *A. tritici* has been recorded infrequently, usually found causing damage to wheat crops (McKay et al., 1952), whereas *A. agrostis* has been reported parasitizing grasses in County Dublin (Farragher, 1975). Little else is known of the occurrence and impact of *Anguina* species on the island but recently, *Anguina*-like galls have been detected on grasses in Irish agricultural advisory samples. These galls have been empty, and to date no *Anguina* nematodes have been found associated with these symptoms. Given the significance of grass fodder and cereal production in Ireland and the potential damage, which could be caused by Anguinid nematodes such as *A. agropyri*, extension nematologists have been undertaking surveillance for these potentially damaging nematodes.

In January 2013, samples of annual meadowgrass/bluegrass (*P. annua*) turf from a golf putting green at a County Dublin golf course in Ireland were sent to The Turf Disease Centre and the AFBINI Plant Pathology diagnostic laboratory for disease analysis. The turf had been displaying symptoms of poor growth for 2 yr, with areas exhibiting chlorosis, progressive turf thinning, and eventually dieback to bare soil. Initially, it was believed that a fungal pathogen may have been the cause of the problem, however, no significant levels of fungal infection were apparent and standard fungicide treatments delivered no improvement in turf health. Given the severity of the damage, it was assumed that the plant parasitic nematode species usually found affecting cool season turf grasses were unlikely to have produced the acute symptoms observed, consequently alternative causes including chemical damage were being investigated. However, close examination of *Poa* plants in the submitted samples revealed root galling and more significantly, the widespread occurrence of blister-like swellings at the base of the plant stems. Basal stem galling is characteristic of two known gall-forming *Anguina* species, *A. agropyri* and *Anguina pacificae* (Cid del Prado Vera and Maggenti, 1984; Subbotin et al., 2004). Here we report on the identification, and discuss the likely origin, of the nematode causing this disease outbreak, the potential risk to agriculture and assess the wider implications for biosecurity in the amenity sector.

Materials and Methods

The site under investigation was a mature 100-yr-old parkland golf course in County Dublin, Ireland. Greens were not the original constructions, having been renovated

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between 20 and 40 yr ago. The site was approximately 12 km from the Irish Sea Coast and symptoms were not apparent on other locations (i.e., fairways, rough) within the course or on greens from other golf courses in the immediate area.

Sample collection: During 2013 and 2014, multiple cores were collected from affected and unaffected areas on greens using a 1.6-cm diameter × 12-cm steel auger. Each sample consisted of at least 12 cores giving a 250 to 300 ml total volume of sample soil. The nematode soil extraction method selected for the survey samples was based on the decanting and sieving Cobb's method, with several modifications to the original protocol (van Bezooijen, 2006). A 100 ml subsample was washed through a 0.5-cm sieve into a bowl and *Poa* plants were collected for microscopic examination. The soil–water mix was transferred into a 1-litre beaker and stirred until a homogenous suspension was obtained. The suspension was then left for 15 sec and the supernatant passed sequentially through 250 µm, 90 µm, and 53 µm sieves. Contents collected in the sieves were poured into a collecting pan lined with an 8-inch easy flow bonded fibre milk filter (GD Textile, Manchester, U.K.) and set in a water-filled glass conical funnel that has a glass vial attached to the bottom opening for collection of nematodes. Samples were left for 24 hr prior to nematode counts and identification. Nematode counts were performed by the addition of water to the collected sample to a total volume of 20 ml. The sample was then briefly vortexed to agitate nematodes into suspension before a subsample was pipetted onto a 1 ml nematode counting slide. The 1 ml counts and nematode identification were taken and the total count for the 100 ml sample was recalculated using a formula that corrected for sample size and soil extraction efficiency. The extraction efficiency for the sieving method was calibrated by performing a comparison to the $MgSO_4$ centrifugal floatation method outlined by Hooper (1986a).

Nematode morphometric analysis: To facilitate the measurement of most morphological characters, adult and J2 nematodes were collected by opening stem galls in tap water. Worms were mounted on microscope slides prepared with a thin layer of water agar over the surface and a coverslip was then placed over the nematodes on the slide. To measure the distance from the anterior end to the junction of the esophagus and intestine, nematodes were killed in hot water (65°C) and fixed in Triethanolamine Formalin (Hooper, 1986b). Nematodes were photographed on a Nikon Eclipse 50i compound microscope and measurements determined using NIS- Elements version 4.10, Nikon imaging software (Nikon, London, U.K.). Prepared specimens were compared morphologically to the published measurements for *A. agropyri* (Cid del Prado Vera and Maggenti, 1984; Chizhov and Berezina, 1986; Brzeski, 1998).

Nematode DNA extractions: Molecular analyses of individual nematodes were performed. The nematode galls were dissected in distilled water from which adult and J2 nematodes were individually picked out, placed on a microscope slide in 20 µl Worm Lysis Buffer (WLB; 50 mM KCl, 10 mM Tris pH 8.2, 2.5 mM $MgCl_2$, 60 µg/ml proteinase K [Roche, Burges Hill, U.K.], 0.45% NP40 [Fisher Scientific, Loughborough, U.K.], 0.45% Tween 20 [Sigma, Gillingham, U.K.], 0.01% gelatine) and cut in half with a scalpel. The nematode and WLB were then transferred into a PCR tube and incubated in a Thermal Cycler (program: 60°C for 60 min, 96°C for 10 min) for DNA isolation. Amplification of the ITS1, 5.8s, and ITS2 regions of the ribosomal DNA cistron was performed using PCR primers, rDNA1 and rDNA2 (Vrain et al., 1992). The PCR assay contained 1 µl DNA, 1 µl rDNA1 primer, 1 µl rDNA2 primer (each at 10 µM), 12.5 µl GoTaq Green Master Mix 2X (Promega, Southampton, U.K.), 9.5 µl H_2O performed with the thermocycler program (program: 94°C for 5 min, 35 cycles; 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and a single 72°C for 5 min). Reference *A. pacificae* J2 nematodes were obtained from Cypress Point GC, California.

PCR purification: Polymerase chain reaction products were purified with the ChargeSwitch PCR Clean-Up Kit (Life technologies, Paisley, U.K.) following the manufacturer's protocol.

DNA sequencing: Sequences were produced for the forward and reverse strands by Queens University Belfast Genomics Core Technology Unit and consensus sequences produced with Geneious version 5.5.6, created by Biomatters (Auckland, New Zealand). The GenBank Accession Numbers for reference sequences: *A. pacificae* Fleming (Dublin) KP715099; *A. pacificae* Powers AF363100; *A. agropyri*, AF396355; *A. agrostis* AM888391; *A. tritici* JF826516; *A. graminis* AF363098.1; *A. phalaridis* AF396352.1; *A. funestra* AF396348.1; *A. woodii* AF363109.1; *A. microlaenae* AF396333.1; *A. caricis* AF396311.1; *A. wevelli* AF396317.1; *A. stipe* AF363106.1; *Subanguina radicicola* AF396366.1; *S. moxae* JN865234.1; *Meloidogyne minor* GU432775.1. The *A. pacificae* McClure sequence was an unpublished partial rDNA reference of a population collected at Cypress Point golf course (courtesy Michael McClure). Likewise, the *A. pacificae* Cypress Point sequence from the Cypress Point golf course population was generated using the rDNA1 and rDNA2 primers to produce a complete reference sequence for comparison with the Dublin population. Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 6 (Tamura et al., 2013).

Host range analysis: The grass and cereal cultivars *Agrostis canina*, *Agrostis tenuis*, *Festuca rubra* cv. Lucinda, *P. annua*, *Poa praten*sis cv. Creon, *Triticum aestivum* var. Einstein, *Triticum aestivum* var. Alchemy, *Hordeum vulgare* var. Suzuka, and *Hordeum vulgare* var. Saffron, were sown in 15-mm diameter pots containing a 9:1 mix of sand and peat. After 2 wk of growth, under a 16-hr light cycle, day temperature ~24°C and night temperature ~16°C, plants were inoculated with a mix of J2 nematodes

hatched in vitro and turf core material containing galls acquired from the infected Golf Course putting greens. 10 wk post inoculation plants were extracted and examined for galls. A total of 6 and 12 replicate plants were examined for the cereal and grass cultivars, respectively.

Results

Disease symptoms on the greens and aprons first appeared as small areas (around 2–4 cm in diameter) of slightly chlorotic turf. Over a period of months, chlorosis increased, the damaged areas gradually expanded (some patches were eventually 3–4 m across), and individual *Poa* plants started to die, eventually resulting in a thinning turf surface. The time from initial symptoms to total turf loss was as little as 6 mon. During 2013, turf loss was evident on five competition greens, a turf nursery, and a practice green. Symptoms in the Irish golf greens were similar to those observed in Californian *P. annua* turf (McClure et al., 2008).

Nematode occurrence: Nematode galling was found on *P. annua* from putting greens and the aprons of the greens. The majority of the galls (92%) were located on the bases of the *Poa* stems, but smaller numbers of galls were also found on roots, as well as on stolons, which are common in this Irish *P. annua* biotype (Christians, 2006). Examination of the structure and contents of root galls suggested that they were the result of *Subanguina* and *Meloidogyne* infection, but levels of root galling were low and unlikely to be causing any significant turf damage. Subsequent DNA sequence analysis of eggs from these galls confirmed the presence of *Subanguina radicicola*, *Meloidogyne naasi*, and *M. minor*. Nematode galls on stems and stolons contained nematode eggs and *Anguina*-like juvenile and adult worms. These galls did not contain the bacteria previously reported to be infecting some *Anguina* galls in Californian samples (McClure et al., 2008).

Morphometrics of stem galling nematodes: Measurements of adult and juvenile *Anguina* from the Irish site confirmed that they were similar to *A. pacificae* and *A. agropyri*, two species also known to produce stem galling in their hosts (Table 1). Most measurements overlapped

Table 1. Summary of morphometrics of Irish *Anguina* and comparison with *Anguina pacificae* and *Anguina agropyri*.

Character[a]	*Anguina* on *Poa annua* Current study (Mean ± SD)	*A. agropyri* Kirjanova, 1955	*A. pacificae* Cid del Prado Vera and Maggenti, 1984 (Mean ± SD)	*A. agropyri* on *Poa annua* Chizhov and Berezina, 1986 (Mean)
Females	*n* = 12	–	*n* = 26	*n* = 23
Body length µm	1,799–2,820 (2184.6 ± 264.3)	1,520–2,420	1,440–2,580 (2,090 ± 120)	1,860–2,560 (2,200)
Body width µm	100.2–124.0 (109.6 ± 7.3)	–	–	–
Ant to o/i µm	154.6–201 (179.5 ± 14.9)	–	–	–
Tail length µm	74.9–110.6 (87.2 ± 9.65)	–	65.2–95.8 (74.9 ± 2.68)	–
V dist µm	1,543–2,318 (1,844 ± 202)	–	–	–
Stylet µm	9.2–12.3 (11.0 ± 0.91)	12	8.9–12.4 (10.8 ± 0.19)	10.2–11.6 (11)
a	15.7–25.5 (20 ± 2.4)	30–36	19.4–33.2 (26.5 ± 1.3)	26.2–35.4 (30.1)
b	9.7–14.2 (12.1 ± 1.21)	–	8.1–13.1 (10.9 ± 0.59)	9.0–14.6 (11.2)
c	19.9–37.1 (25.8 ± 4.6)	22–28	19.9–34.4 (27.8 ± 1.7)	16.6–21.5 (18.6)
V%	80.3–89.5 (84.7 ± 2.4)	78–80	82–89 (86 ± 1.8)	74–84 (80)
Males	*n* = 12	–	*n* = 17	*n* = 22
Body length (µm)	1,503–1,871 (1,693 ± 131)	1,400–1,800	1,220–1,840 (1,560 ± 75)	1,470–2,320 (1,890)
Body width (µm)	62.5–77.3 (68.7 ± 5.44)	–	–	–
Ant to o/i µm	148.9–197.6 (167.8 ± 18.7)	–	–	–
Tail length (µm)	71.6–85.3 (80.5 ± 4.72)	–	58.3–76.9 (68.7 ± 2.7)	–
Spicules (µm)	29.9–43.8 (37.2 ± 4.6)	30–35	26.2–40.7 (34.2 ± 2.7)	26–33(29)
Gubernaculum (µm)	11.6–15.6 (13.2 ± 1.2)	15	10.6–15.5 (13 ± 0.61)	8.7–14.5 (11.2)
Stylet (µm)	9.7–12.7 (11.2 ± 0.89)	12	9.3–12.7 (11 ± 0.46)	10–12 (11)
a	23.1–28.2 (24.7 ± 1.63)	50–60	21.1–33 (27.9 ± 1.71)	22.2–36.3 (28.6)
b	8.2–12.1 (10.2 ± 1.2)	–	5.8–10.4 (9.1 ± 0.79)	7.4–13.1 (9.2)
c	19.3–23.5 (21 ± 1.44)	14–19	19.8–29.8 (23.2 ± 1.25)	14.1–22.9 (17.9)
Juveniles	*n* = 14	–	*n* = 20	*n* = 20
Body length (µm)	590–864 (711.6 ± 64.9)	830–950	630–970 (710 ± 43)	780–1,240 (1,000)
Body width (µm)	17.2–20.1 (18.3 ± 1)	–	–	–
Ant to o/i µm	104.8–184.7 (161.5 ± 18.4)	–	–	–
Tail length µm	73.1–85.6 (78.5 ± 4.1)	–	58.6–82.4 (70.4±3.2)	–
Stylet (µm)	10.3–12.6 (11.2 ± 0.6)	9	10–12.7 (11 ± 0.35)	9–12.2 (11)
a	32.8–44.3 (38.8 ± 3.2)	53.5–63.3	37–56.4 (43.8 ± 2.1)	49–68 (56.4)
b	3.7–6.6 (4.4 ± 0.7)	–	3.5–7.3 (4.5 ± 0.41)	4.5–5.6 (5)
c	6.9–11.4 (9.1 ± 1)	–	8.8–11.4 (9.9 ± 0.35)	10.4–12.2 (10.9)

[a] Character: a = body length ÷ greatest body width, b = body length ÷ distance from anterior end to junction of esophagus and intestine (o/i), c = body length ÷ tail length (anus or cloaca to tail terminus), V% = distance of vulva to anterior end × 100 ÷ body length, Ant to o/i = distance from anterior end to dorsal esophageal intestinal gland outlet.

for the three *Anguina* isolates but a number of characters, such as adult a, female c, and V%, indicated a closer identity between the Irish isolate and *A. pacificae*. The Irish isolate also exhibited the sharply pointed tail characteristic of *A. pacificae*, and the adult male bursa ended before the tail tip, which is also a feature of the Californian species.

Molecular diagnosis: The DNA sequences generated from the Dublin *Anguina* specimens were initially compared through aligments to previously reported sequences of *A. agropyri* and *A. pacificae*, as well as several other *Anguina* spp., *A. agrostis*, *A. graminis*, *A. tritici*, *A. phalaridis*, *A. funestra*, *A. woodii*, *A. microlaenae*, and *A. caricis*. *Meloidogyne* and *Subanguina* species were included in analysis to allow a comparison between the *Anguina* species. In addition, an unidentified *Anguina* sp. sequence was included from a population collected from the Lake District, United Kingdom. These revealed the Dublin *Anguina* nematodes were most similar to *A. pacificae*. The previously reported reference sequences for *A. pacificae* were not as extensive and did not span the entire length of the Dublin *Anguina* sequence fragment. Therefore, *A. pacificae* J2 nematode specimens from Cypress Point Golf Course, CA were obtained to produce a complete reference sequence for the ITS1, 5.8s, and ITS2 regions of the ribosomal DNA cistron, therefore, confirming complete homology with the Dublin *Anguina* nematodes (Fig. 1). The Dublin *Anguina* rDNA sequence percentage similarity was 100% with the three *A. pacificae* sequences, whereas the similarities for *A. agropyri*, *A. graminis*, *A. agrostis*, and *A. tritici* were 97%, 95%, 94%, and 93%, respectively. Furthermore, the Lake District *Anguina* sp. sequences showed no match to any of the *Anguina* spp. included, and were distantly related to that of any of the common European *Anguina* spp.

Anguina stem galling: To assess the levels of infection in three affected competition greens, *Poa* plants from nonsymptomatic turf, the leading edge of infected patches, and the center of patches were examined individually and infection levels were determined (Table 2). *Poa* plants from nonsymptomatic areas contained less than 5% infection, whereas infection rates in patches ranged from 21% to 75%, with plant density greatly reduced in the patches as a result of *Poa* mortality.

Seasonal population dynamics: Periodic sampling of infected turf showed that numbers of adult male and female *A. pacificae* in galls varied during the year, with

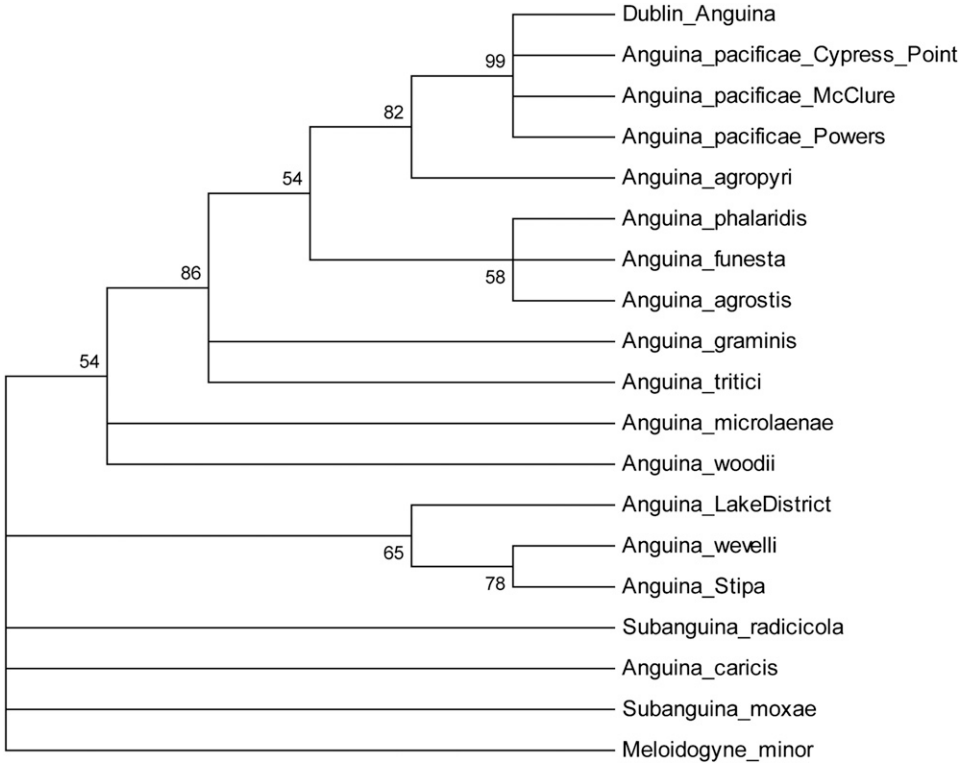

Fig. 1. Molecular Phylogenetic analysis of *Anguina* spp. using rDNA reference sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). The bootstrap consensus tree inferred from 1,000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.8695]). The analysis involved 19 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 321 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

TABLE 2. *Anguina pacificae*. Levels of galling associated with symptomatic and asymptomatic turf in three affected greens: expressed as the percentage of *Poa* plants with galls (total number of *Poa* plants in the 100 cm^3 sample).

	Healthy turf	Edge of patch	Centre of patch
Green 1	5% (260)	54% (24)	28% (43)
Green 5	1% (202)	72% (56)	21% (67)
Green 16	0% (221)	40% (70)	75% (60)

peak numbers in June and July (Fig. 2). Generally in the examined galls, males outnumbered females and overtime adults appeared to die and decompose within the galls, leaving only developing eggs and juveniles. Infective stage juvenile numbers in the soil were low throughout most of the year but rose sharply in August 2013 and again were elevated in October and November 2014 (Fig. 3).

Host range: Several grass and cereal cultivars were tested for susceptibility to the *A. pacificae*, Dublin population. Of all the cultivars examined, only the *P. annua* and Velvet bentgrass (*A. canina*) had observable stem galls containing viable eggs. The other cultivars examined showed no galling; *P. pratens*is cv. Creon, Highland browntop bent (*A. tenuis*), *F. rubra* cv. Lucinda, *Triticum aestivum* var. Einstein, *Triticum aestivum* var. Alchemy, *Hordeum vulgare* var. Suzuka, and *Hordeum vulgare* var. Saffron.

Climate data: For Dublin (Ireland), the total annual precipitation is 729 mm and average annual high and low temperatures are 12.8°C and 6.4°C, respectively (EU Climate Data), whereas in Monterey, CA, total annual precipitation is 537 mm and average annual high and low temperatures are 17.5°C and 9°C, respectively (US Climate Data).

Discussion

The disease symptoms from plant specimens collected from the infected golf greens were diagnosed by both morphological and molecular analysis of the nematodes extracted from the soil and basal stem galls. Given the characteristic basal stem galls in the infected grass, the two *Anguina* species known to display were *A. agropyri* and *A. pacificae*. Initial morphological diagnosis noted distinct annulated tail tips of the second stage juveniles, most similar to previous reports of *A. pacificae*. The molecular analysis of the Dublin *Anguina* later confirmed that the specimens were in fact *A. pacificae*. The phylogenetic relationship of the Irish *A. pacificae* isolate sequences was compared with previously confirmed sequences and that of other *Anguina* species. The rDNA analysis, previously successful for distinguishing *Anguina* spp. (Powers et al., 2001), confirmed complete sequence homology with the *A. pacificae* sequences, any minor differences detected were present at the ends of sequences and thereby deemed sequencing

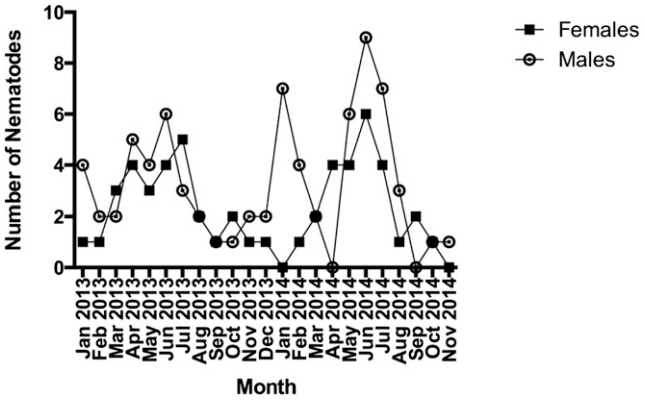

FIG. 2. Numbers of male and female *Anguina pacificae* per gall (mean of three galls) from periodic samples collected at the Dublin golf course.

error. Notably, the model strength was validated by the position of *A. agropyri* to *A. pacificae*. In addition, the Irish population successfully reproduces and completed a full lifecycle in *P. annua*. After the climate data from each of the regions were compared, it was noted that precipitation was higher in Dublin but experienced annual average temperatures 2°C to 5°C lower than in Monterey, CA.

Identification of *Anguina* species using morphological characters can be difficult, with information on the host plant the nematode is parasitizing, often crucial to the diagnosis. In particular, body measurements and proportions can be affected by factors such as the developmental state of the nematode, host nutritional factors, and method of sample preparation (Brzeski, 1981; Southey et al., 1990). The confirmation of *A. pacificae* in Ireland was, therefore, based on a combination of data types including host symptoms, morphology, and DNA sequences. *A. pacificae* has both a restricted host

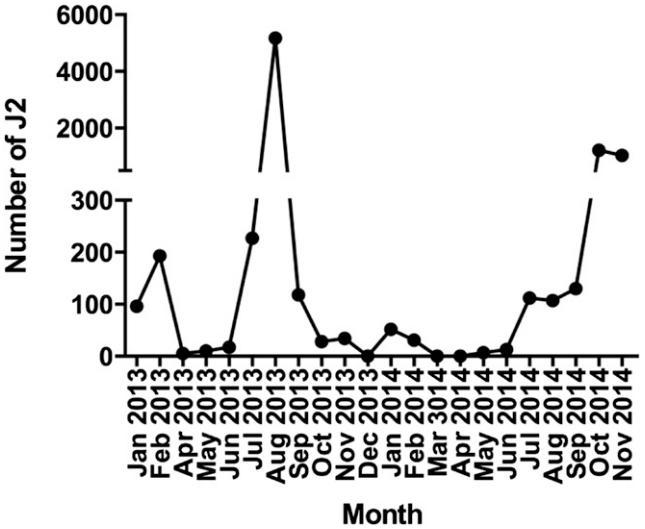

FIG. 3. Number of *Anguina pacificae* juvenile numbers (mean of 3 x 100 cm^3 soil) in samples from infected *Poa* turf collected at the Dublin golf course.

range and geographic distribution and has not been recorded on North American sports turf outside its apparent natural range in northern California (McClure et al., 2008). Vandenbossche et al. (2011) surveyed the plant parasitic nematodes in Belgian turfgrass and did not detect any *Anguina* species, while the leaf gall forming species *A. graminis* has occasionally been found in turfgrass in Britain and Switzerland. Most recently in 2014, an undescribed *Anguina* species was found parasitizing bentgrass leaves and causing severe damage in a bowling green in the Lake District region of England. This recent detection of an *Anguina* population with a distinct sequence homology to that of any other reported species illustrates there are potentially many unreported species still to be characterized. In the case of Ireland specifically, two recent and extensive surveys did not detect any *Anguina* spp. from 191 samples collected from agricultural grassland between 2011 and 2013 (Fleming, 2015) or from over 500 advisory samples of Irish sports turf analyzed between 2009 and 2014 (C. Fleming, unpublished).

Discovered in California, *A. pacificae* was initially identified as *Anguina radicicola* (Costello, 1983), however, on the basis of morphological data, Cid del Prado Vera and Maggenti (1984) proposed that the nematode was in fact a new species. The status of *A. pacificae* came into question when a suspected *A. agropyri* (*Paranguina agropyri*) population detected on *P. annua* in the Moscow region of Russia and producing *A. pacificae*-like galls, was found to be morphologically very similar to *A. pacificae* (Chizov and Berezina, 1986). Chizhov and Subbotin (1990) thus considered *A. pacificae* to be a synonym of *A. agropyri*. Subsequent molecular analyses demonstrated European *A. agropyri* and Californian *A. pacificae* to be genetically distinct (Subbotin, pers. comm.) but in the absence of molecular data, there is still some doubt as to the identity of the Moscow *Anguina* population. Assuming the morphometric data to be definitive, Subbotin considered that *A. pacificae* may have a much wider distribution than simply the coast of northern California and raised the possibility that it is also native to Russia and perhaps elsewhere within Europe. This study is the first confirmed report of *A. pacificae* outside its presumed natural range along the coast of northern California, where populations cause significant damage on *P. annua* turfgrass (Westerdahl et al., 2005). Given the severe turf damage observed in Ireland, it is important to determine if this disease is a result of an accidental introduction of *A. pacificae* into Ireland from the United States or if it represents the emergence of *A. pacificae* as a previously unrecorded native European pest, which may pose a significant and wider threat to the European golf industry. *P. annua* is the dominant grass species in golf greens throughout the British Isles (Baker et al., 1995), however, *A. pacificae* damage symptoms have not been previously apparent elsewhere in the United Kingdom and Ireland which would add weight to the argument that *A. pacificae* is not native to the British Isles.

As this appears to be the first confirmed report of *A. pacificae* outside northern California, there was a necessity to assess the species pathogenicity and host range for the local amenity and agricultural plant varieties, given the severity of damage caused by the closely related species, *A. agropyri* in eastern European cereals. It is important to determine whether the population has been transferred to the Irish site by contamination or whether this population could be native. After weighing the evidence, it seems more likely that the current case may be a result of an accidental introduction from North America. Two possible sources of infection could be contaminated machinery or possibly player's equipment such as golf shoes. Given that *A. pacificae* has been shown to survive extended periods of anhydrobiosis, transfer in dried plant material is a likely route of entry for the nematode (McClure et al., 2008). As for the potential spread of the pest species to Irish Agriculture, the threat appears to be minimal due to *A. pacificae*'s relatively limited host range compared to many of the common *Anguina* species that parasitize plants including many agriculturally important crops (Table 3). However, as Irish golf courses predominantly use *P. annua* turfgrass and *A. pacificae* can survive extended periods in a desiccated state, the spread of this particular pest is of real concern for turfgrass industries. Furthermore, the lifecycle dynamics of the Irish populations showed similar trends to those of California populations (Giat et al., 2008), such as the single large J2 hatch that was observed in August 2013. In the following year, levels were much lower, attributed to the extensive nematode control strategy in place after the initial confirmation.

Biosecurity issues in sports turf: The discovery of *A. pacificae* in Ireland highlights the growing significance of biosecurity in the amenity sports turf industry. The increasing use of sand-based root zones for sports pitches and golf constructions in northwest Europe has contributed to the development of nematode problems in turf grass production and maintenance. In many cases, turf sod and construction materials appear to be the initial source of nematode infection in new pitches and greens (Entwistle et al., 2014). In particular, *M. naasi*, *M. minor*, and *Meloidogyne fallax*, and *Hemicycliophora conida* (sheath nematode) have all been found causing serious turf damage in ryegrass (*Lolium perenne*) sports fields across the United Kingdom, with infected sand from sand extraction sites within the United Kingdom the likely source of these nematodes. Interestingly, *M. fallax*, an A2 European Plant Protection Organization (EPPO) quarantine species, and initially believed to be absent from the British Isles, was detected in a number of soccer pitches in the London area. Construction sand for these pitches had been sourced from the same sites leading to the suspicion that this species was in fact native to the United Kingdom. Subsequently,

TABLE 3. List of host range and geographical distributions of selected *Anguina* spp.

Species	Host range	Distribution
Anguina tritici	*Triticum aestivum* L.	Asia
	Triticum dicoccum Shrank	Australia
	Triticum durum Desf.	Europe
	Triticum monococcum L.	North Africa
	Triticum spelta L.	North America
	Triticum ventricosum Ces.	
	Secale cereale L.	
	Hordeum sp. (poor host)	
	Avena sp. (poor host)	
Anguina agrostis	*Agrostis tenius*	Asia
	Apera sp.	Europe
	Arctagrostis sp.	North America
	Calamagrostis sp.	Australia
	Dactylis sp.	New Zealand
	Eragrostis sp.	South Africa
	Festuca sp.	
	Hordeum sp.	
	Koeleria sp.	
	Lolium sp.	
	Phalaris sp.	
	Phleum sp.	
	Poa sp.	
	Puccinellia sp.	
	Sporobolus sp.	
	Trisetum	
Anguina agropyri	*Agropyron repens*	Europe
	Secale cereale L.	
	Triticum vulgare L.	
	Hordeum sativum L.	
	P. annua L.	
	Elymus arenarius L.	
Anguina pacificae	*P. annua* L.	North America
	Agrostis canina	

during 2013, *M. fallax* was found in a leek (*Allium ampeloprasum*) crop in England (Fera) supporting the view that the nematode is in fact endemic but rare within Britain, and that other unreported species may be endemic.

Although *M. naasi* is the most common root knot nematode found in U.K. turf grass, *M. minor* is emerging as a cause of serious turf damage, especially in creeping bentgrass (*Agrostis stolonifera*) USGA golf green constructions, where damaging levels of the nematode can develop within 18 mon of seeding a new green. This species has been introduced into new green complexes in construction sand and sports constructions sourcing material from the same sites tend to contain similar plant parasitic nematode communities. The detection of *M. minor* on two golf courses from Washington State may indicate a recent accidental introduction of this species into the United States from Europe (McClure et al., 2012).

P. annua dominates golf greens within the British Isles (Baker et al., 1995) and the primary sources of nematode damage in this turf species are the spiral nematode *Helicotylenchus pseudorobustus* and the root gall nematode, *S. radicicola*. *S. radicicola* damage usually results in small patches of chlorotic turf, which exhibit poor turf density and weak root systems (Mitkowski and Jackson, 2003) and turf instability resulting from *S. radicicola* damage has actually led to the cancellation of a U.K. horse racing event on the grounds of health and safety (BBC, 2010). The appearance of *A. pacificae* on an Irish golf course demonstrates the potential for movement of pests and pathogens in an increasingly international amenity sector.

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