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Short Communication

Ultrastructural Characterization of *Meloidogyne graminis* from Golf Course Turf Grasses in Peninsular Malaysia

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ABSTRACT

During year 2015, a survey was conducted in 18 golf course greens from nine states (Selangor, Negeri Sembilan, Melaka, Johor, Pahang, Perak, Kedah, Pulau Pinang and Wilayah Persekutuan) of Peninsular Malaysia for nematode, *Meloidogyne graminis* infections on the turf grasses. Samples were collected based on the sparsely growth and chlorotic appearance of the greens. A total of 36 soils and roots sample were collected. Scanning electron microscopy (SEM) was used to identify the parasitic nematode. Both the field symptoms and SEM micrographs confirmed that the nematode isolate was *M. graminis*. Since this nematode has been known to damage the greens and other plants in other part of the world then the probability for the specie to adapt to other hosts other than the family of poaceae under Malaysian climate should not be discounted. Therefore, screening and restriction of movement of planting materials be observed critically.

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Authors' Contribution
SB conducted the survey. IAG assisted in survey and processing of samples. JK designed and supervised the research. ASP cultured *Meloidogyne graminis*. YI and MK helped in writing the article.

Key words
SEM, *Meloidogyne graminis*, Infection, Turf grass.

Health, quality, production, and maintenance of turf grass on golf courses have been affected significantly by plant-parasitic nematodes. By infecting the root system, plant parasitic nematodes (PPN) influence the physiological processes of the entire plant, either directly or indirectly. Nematodes affect plant growth by disrupting cell structure, removing cell contents, altering metabolism and modifying the genetic expression of the host (Bongers and Bongers, 1998; Milesi *et al.*, 2005; Cheng *et al.*, 2008). As PPN feed on plant parts, subsequent damage is reduces the ability of root systems to uptake water and nutrients from the soil solution (Khan, 1993). Infestations of PPN frequently cause turf grasses to become more susceptible to environmental stresses. Parasitized roots may be shortened and appear darkened or rotted (Crow and Welch, 2004). Roots may also exhibit knots or galls and/or display excessive branching (Blake, 1999). When nematode population densities become high enough, and/or environmental stresses such as high temperatures or drought occur, above ground symptoms usually become detectable as a result of this stunted growth and discoloration. Foliar symptoms include yellowing, wilting, browning, thinning out, poor response to fertilization and irrigation, or death of grass. Damage often occurs as irregularly shaped chlorotic patches that may enlarge in diameter over time (Crow and Grewal, 2009). More than 20 genera of PPNs are known to actively parasitize and cause damage to turf grasses (Dunn and Diesburg, 2004). The most common of these twenty nematode genera include lance, *Hoplolaimus galeatus* (Giblin-Davis *et al.*, 1995), ring, *Criconemella* spp. (Crow *et al.*, 2009), root-knot, *Meloidogyne* spp. (Starr *et al.*, 2007), spiral, *Helicotylenchus* spp. (Subbotin *et al.*, 2011), sting, *Belonolaimus longicaudatus* (Bekal and Becker, 2000a, b), stubby-root, *Paratrichodorus* and *Trichodorus* spp. (Crow and Welch, 2004) and stunt, *Tylenchorhynchus* spp. (Mai and Lyon, 1975). Of these nematodes, sting and stubby-root tend to cause the most severe damage to turf grass (Schwartz *et al.*, 2010; Wetzel, 2000). Turf grasses that are hosts for these nematodes include Bermuda grass, bentgrass

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(Sikora *et al.*, 1999), zoysia, tall fescue (Nyczepir, 2011), seashore paspalum (Ye *et al.*, 2012), bluegrass (Coates-Beckford and Malek, 1982), ryegrass (Griffin *et al.*, 1984) and switch grass (Cassida *et al.*, 2005). Root-knot nematodes, *Meloidogyne* spp. were frequently associated with turf grass in Malaysia, alongside, *Xiphinema* spp., *Hoplolaimus* spp., *Pratylenchus* spp., and *Criconemoides* spp. (Rahman and Evan, 1988).

Current research was aimed to investigate characterization of *Meloidogyne graminis* using electron microscopy as it is thought to be able to help in devising a control strategy associated with Golf course turf in Malaysia.

Materials and methods

In 2015, survey was conducted in 18 golf course greens from 9 states (Selangor, Negeri Sembilan, Melaka, Johor, Pahang, Perak, Kedah, Pulau pinang and Wilayah Persekutuan) of Peninsular Malaysia for *M. graminis* infections on the turf grasses. Samples were collected based on the sparsely growth and chlorotic appearance of the greens. A total of 36 soils and root samples were collected following the sampling procedure proposed by Speijer and De Waele (1997). Roots were separated from soil, washed and stained by boiling in acid fuschin lactophenol, following Hooper (1986). Root galls with matured females were cut into 1cm length, fixed in formalin acetic acid, post fixed in 1% buffer osmium tetraoxide and then dehydrated through sequence of graded ethanol to absolute ethanol. The samples were then dried in critical point drier (CPD) Balzar 030, mounted on stub and splutter gold coated and viewed under JOEL 6400 at accelerated voltage of 15 kv.

Results

Saccate *M. graminis* females were observed partially embedded in the root tissue (Fig. 1A). Manifest symptoms of infested roots were slight enlargement of the root where the female nematode resides. The extent of body exposure outside the root tissues is influenced by the shape of the swollen mature female. For most of the oval shaped females, lateral posterior half of the body is seen outside the root while the remaining half of the body is embedded in the cortical tissue of the root. However, the cortical tissue around the body of the nematode seems to split longitudinally to accommodate the swollen mature female (Fig. 1B). Only the head and neck of the spherical females were found embedded in the root tissue, the enlarged part of the body remaining outside the root in egg laying females, the exposed posterior part of the body was generally covered by eggs and gelatinous matrix (Fig. 1B).

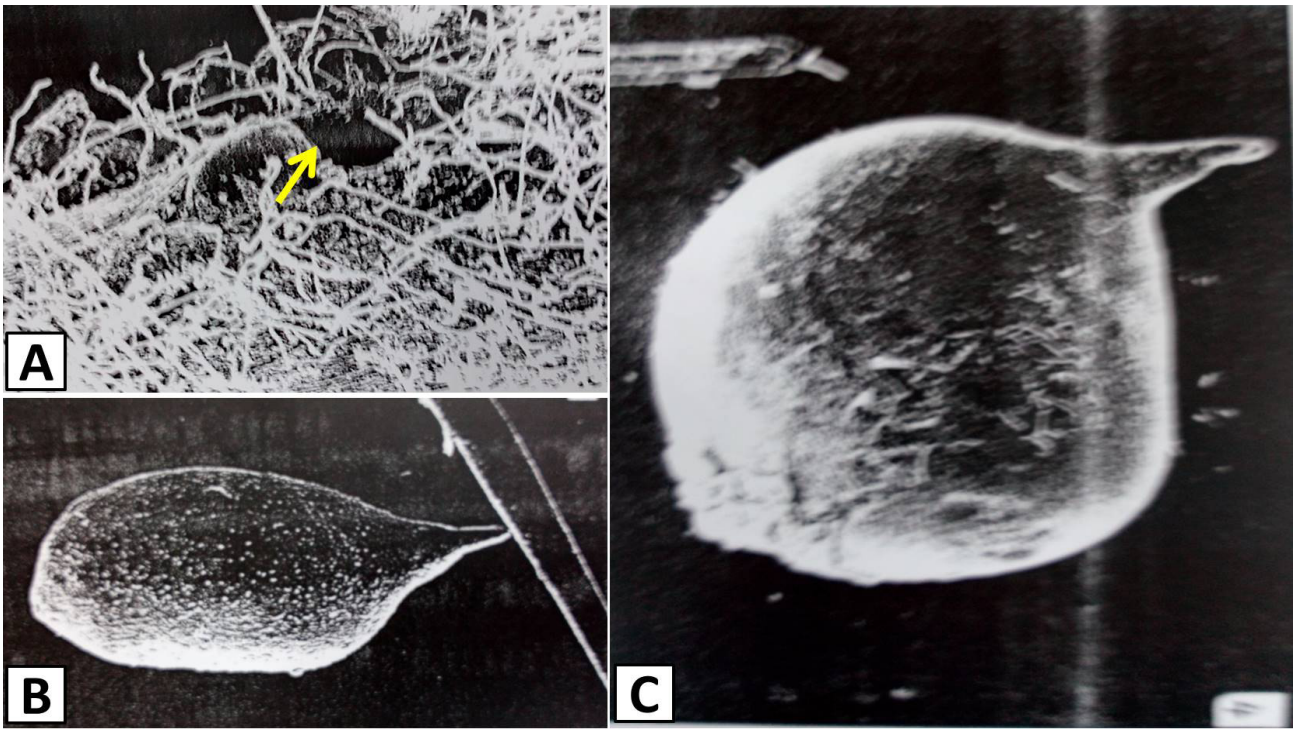

Fig. 1. Turf grass root infected by female (A) and an oval-shaped saccate female (B) of *Meloidogyne graminis*. C, oval shaped female detached from root tissues. The head is on the opposite side of perineal pattern along the body axis.

The oval-shaped females have their vulva and anus located on protuberance on the same longitudinal axis of the body at opposite end of the head (Fig. 1C). Contrary, the spherical shaped females have their head and neck protruded to only one side of the body, at an angle with longitudinal axis of the body (Fig. 2).

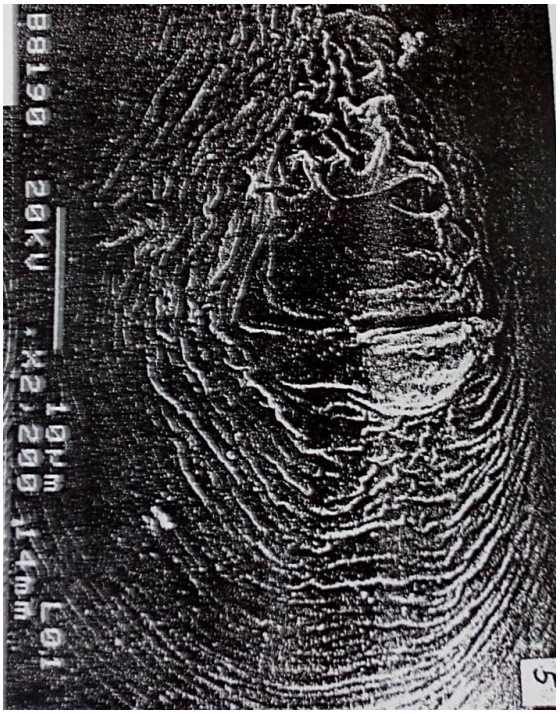

Fig. 2. Spherical-shaped body of the saccate female of *Meloidogyne graminis*. The neck region is almost at right angle to the longitudinal body axis.

The perineal pattern (posterior cuticular pattern) of the nematode was found to be oval shaped, possessing trapezoidal dorsal arch. The striae were smooth or slightly wavy with prominent lateral lines (Fig. 3).

Discussion

Of the 18 golf courses sampled, *M. graminis* was found in 14 golf courses across peninsular Malaysia. Our results reveal how widely distributed the parasite is on golf course greens in Peninsular Malaysia. Our findings corroborate with studies from other workers (Grisham *et al.*, 1974; Vandenbossche *et al.*, 2011; McClure *et al.*, 2012) who reported *M. graminis* as an economically important species to golf course industries through decline in turf quality by causing the grass to be chlorotic, stunted in growth and at some levels may cause the plant to die. Similarly, Hunt and Handoo (2009) found that the most economically damaging nematode species on horticultural

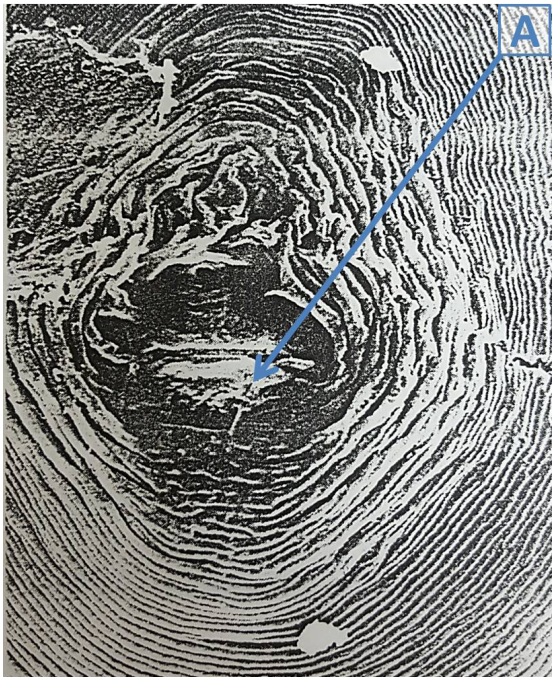

Fig. 3. A side view of the vulva and anus on protuberance at the posterior terminal of the body.

and field crops are the root knot nematodes. Having a worldwide distribution, and being obligate, make them parasites of the roots of many plant species, of monocots and dicots, and woody and herbaceous plants. Obvious sign of RKN infection are root galls, shoot chlorosis, stunted growth, nutrient deficiencies, and paving way for secondary infections by other pathogens (Hunt and Handoo, 2009). A high level of damage can lead to total crop loss. So far, there are nine species of *Meloidogyne* associated with turf grasses worldwide, including *M. graminis* Sledge and Golden Whitehead, *M. chitwoodi* Golden, *M. incognita* Kofoid and White, 1919, *M. fallax*, *M. Graminicola* Golden and Birchfield, *M.* marylandi Jepson and Golden, *M. Microtyla* Mulvey, *M. minor* Karssenand *M. naasi* Franklin (Crow, 2005; Vandenbossche *et al.*, 2011; McClure *et al.*, 2012). This is the first time this nematode is reported in Malaysia. There is every possibility that the nematode could have been transported with the stolon planting materials from United States. However, the probability for the specie to adapt to other hosts other than the family of poaceae under Malaysian climate should not be discounted. Therefore, screening and restriction of movement of planting materials be observed critically.

Statement of conflict of interest

The authors declare no conflict of interest.

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