

First Report of *Scutellonema brachyurus* (Steiner, 1938) Andrassy, 1958 and Occurrence of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 in Belgium

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Recently, the presence of tropical nematodes has been reported from several Mediterranean or more southern European countries (Wesemael et al., 2011; EPPO, 2019). However, tropical nematodes do not yet pose a problem in the more northern parts of Europe, including Belgium. According to Bebbier et al. (2013), there is clear evidence of a general, climate change-driven, poleward migration of pests and plant pathogens, a movement that can include tropical plant-parasitic nematodes. Interestingly, our survey revealed the presence of *Scutellonema brachyurus* and the tropical root-knot nematode *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 for the first time, respectively, on banana (*Musa basjoo* Siebold & Zucc. ex Linum) and red ginger (*Hedychium greenii* W. W. Sm.) in Belgium.

Abstract

A study of plant-parasitic nematodes in the Botanical garden at Ghent University in Belgium revealed the presence of two tropical nematode species, i.e. *Scutellonema brachyurus* and *Meloidogyne incognita*. *Scutellonema brachyurus* was recovered, only once, for the first time in Belgium from *Musa basjoo* and is morphologically characterized. *M. incognita*, forming galls on *Hedychium greenii*, was recovered in all seasons over three consecutive years and is morphologically and molecularly characterized. Although no unequivocal evidence was found to indicate that these nematodes pose a current threat in Belgium, in the light of climate change, it is crucial to improve our knowledge of potential tropical nematode activity in more Northern countries.

Keywords

Global warming, *Hedychium greenii*, *Musa basjoo*, mtDNA, *Nad5*.

Materials and methods

After removing detritus layer from the surface, soil and root samples were collected from the upper 30 cm soil layer around the rhizosphere of *Musa basjoo* and *Hedychium greenii* at the Botanical garden of Ghent University (GPS coordinates: N: 51°2'6.8", E: 3°43'22.7" and N: 51°2'6.7", E: 3°43'22.4", respectively). Several samples were taken from September 2017 to June 2019 to check for the survival of nematodes through the winter season. For morphological characterization, vermiform nematodes were extracted by the modified Baermann tray method (Whitehead and Hemming, 1965). After that, permanent slides were made following Singh et al. (2018). Mature females of *M. incognita* were extracted directly from the galls

under a stereomicroscope, using a scalpel and forceps. Perineal patterns were cut and cleaned following Hartman and Sasser (1985) and mounted in glycerine. Microphotographs were made from permanent slides using an Olympus BX51 DIC Microscope equipped with a digital camera. Measurements were made based on the obtained pictures using Image J 1.51 (Schneider et al., 2012). For molecular characterization, primers NAD5F2/NAD5R1 were used to amplify the *Nad5* mtDNA gene following the protocol of Janssen et al. (2016). *Nad5* mtDNA sequences of *M. incognita* in Belgium were aligned with 73 reference sequences of tropical root-knot nematode species from Janssen et al. (2016) and other closely related sequences from GenBank using Muscle on Geneious R11 (www.geneious.com) to check for polymorphic nucleotide positions.

Results

Scutellonema brachyurus (Steiner, 1938) Andrásy, 1958

(Fig. 1, Table 1).

Remarks

Morphological traits and measurements of the female Belgian population of *S. brachyurus* is in full agreement with the type population of Steiner (1938), except for the slightly shorter stylet (26.8 ± 1.1 (25.2–28.6) μm vs 29 μm). However, this variation is known in other populations of *S. brachyurus* (Table 1). Van Den Berg et al. (2013) described two types of *S. brachyurus* (type A and type B), and *S. brachyurus* type A can be differentiated from type B by the main following traits: lip region with 4 to 6 annuli vs mainly three, rarely 4 to 5 annuli; 4 to 12 blocks on basal annulus vs 8 to 20 blocks; secretory–excretory pore located opposite anterior part to mid-region of overlapping pharyngeal lobe vs from rarely opposite mid-isthmus to mostly opposite the posterior part of pharyngeal gland lobe up to its posterior border. The Belgian population of *S. brachyurus* belongs to *S. brachyurus* type B. Unfortunately, our effort to recover the Belgian population of *S. brachyurus* for molecular analysis was unsuccessful. Males were also not found.

Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949

(Fig. 1, Table 1).

Remarks

In general, the morphology of *M. incognita* in Belgium is in agreement with the description of Whitehead (1968) (the perineal pattern of Belgian population of *M. incognita* belongs to *M. incognita incognita* type). Only a few variations of measurements were observed such as a wider upper range of body length of juveniles (406 (374–420) μm vs 371 (337–403) μm) and a larger DGO value of the males (3.8 (3.2–4.4) vs 2.1 (1.4–2.5)). However, these variations are small and fall within the range known from other populations (Table 1). Six *Nad5* sequences were obtained with a length from 544 to 599 nucleotides. The sequences of *M. incognita* in Belgium were identical to each other and five other reference sequences of *M. incognita* (specimen ID: T384, T532, M8, M20, M21) from Morocco, Egypt, and Tanzania in the study of Janssen et al. (2016). During winter time, the aerial parts of the host plant were cut down and the growing area was covered by wood chips. *Meloidogyne incognita* has been found at any time of sampling in all seasons over three consecutive years.

Discussion

This study reveals the presence of *S. brachyurus* and *M. incognita* in Belgium, species known to be prevalent in warm areas, especially in tropical regions (CABI, 2019). Morphologically, the Belgian population of the spiral nematode belongs to the *S. brachyurus* group type B according to Van Den Berg et al. (2013). However, the presence of cryptic species in the *S. brachyurus* group have only been molecularly unequivocally defined (Van Den Berg et al., 2013; Van Den Berg et al., 2017), and therefore, molecular data are needed to confirm the group of *S. brachyurus* to which the Belgian population belongs. Unfortunately, multiple attempts to recover this nematode for molecular studies failed.

The morphological identification of tropical root-knot nematodes is known to be greatly hampered by phenotypic plasticity and interspecific similarities (Hunt and Handoo, 2009), for example the variation in the number of post-labial annuli of *M. incognita* in Belgium (ranging from 1 to 3 annuli) confirmed the plasticity of the numbers of head annuli in the genus *Meloidogyne*. Therefore, we used an integrated approach including the mitochondrial barcode region *Nad5* as a reliable marker to identify tropical root-knot nematodes.

The Belgian population of *M. incognita* was discovered for the first time on a specimen of *Hedychium greenii* that was planted outside several years ago

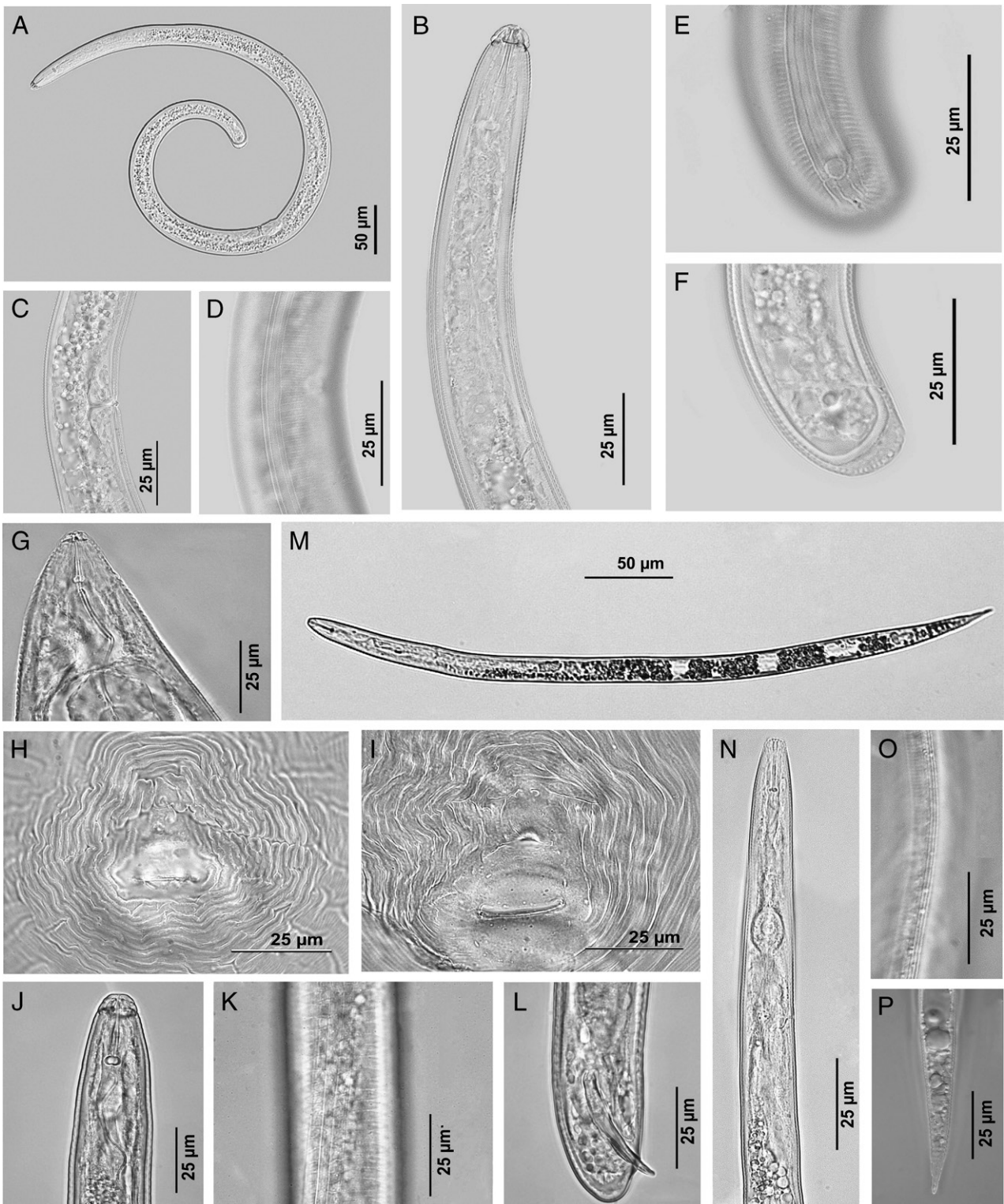


Figure 1: LM pictures. (A–F) females of *Scutellonema brachyurus* from Belgium. (A) Entire body; (B) Anterior region; (C) Vulva region; (D) Lateral field at vulva region; (E) Scutellum at tail region; (F) Tail region. (G–P) *Meloidogyne incognita* from Belgium. (G–I) Mature females. (G) Anterior region; (H, I) Perineal patterns. (J–L) Males. (J) Anterior region; (K) Lateral field; (L) Tail region showing copulatory apparatus. (M–P) Second-stage juveniles. (M) Entire body; (N) Anterior region; (O) Lateral field; (P) Tail region.

Table 1. Measurements of *Scutellonema brachyurus* and *Meloidogyne incognita* from Belgium

	<i>S. brachyurus</i> (Belgium)		<i>M. incognita</i> (Belgium)	
	Females	Juveniles	Males	Females
<i>n</i>	11	20	10	10
Body length (L)	699±50 (625–774)	406±17.1 (374–420)	1884.0±135 (1728–2048)	584±66.8 (506–751)
<i>a</i> = L/MBD	28.3±7 (23.8–44)	27.4±2.4 (23.5–32.6)	48.5±4.0 (44.9–54.2)	1.8±0.1 (1.5–2.0)
<i>b</i> = L/anterior to pharyngo-intestinal valve	6.6±0.44 (5.9–7.2)	–	–	–
<i>b'</i> = L/ anterior to base of pharyngeal gland	5.5±0.42 (4.9–6.1)	3.0±0.3 (2.4–3.5)	7.9±0.6 (7.3–8.6)	–
<i>c</i> = L/Tail length	79±44 (49–177)	8.4±0.4 (7.7–9.0)	204.4±107.6 (137.1–365.1)	–
<i>c'</i> = Tail length/ABD	0.66±.06 (0.55–0.76)	5.0±0.4 (4.5–5.6)	0.4±0.3 (0.1–0.6)	–
V%	59±1.5 (57–62)	–	–	–
Distance from anterior end to middle of genital primordium	–	254±18.2 (228–279)	–	–
Lip height	5.7±0.4 (5.1–6.2)	–	7.2±1.1 (6.3–8.8)	–
Lip width	9.1±0.37 (8.7–9.6)	–	13.4±0.3 (13.2–13.9)	–
Stylet length	26.8±1.1 (25.2–28.6)	10.9±0.8 (9.5–12.0)	24.7±0.9 (23.9–25.8)	15.6±0.7 (14–17)
Conus length	14.1±1.1 (12.7–15.8)	6±0.5 (5–6)	13.9±0.5 (13.2–14.5)	9±0.9 (8–10)
Shaft length	10.1±0.39 (9.5–10.5)	4±0.6 (3–5)	8.0±0.6 (7.6–8.8)	4.8±0.7 (4–6)
Knob height	2.7±0.39 (2.3–3.4)	1±0.0 (1–1)	2.8±0.4 (2.5–3.2)	2.3±0.3 (2–3)
<i>m</i> = Cone/Stylet	0.52±0.03 (0.48–0.57)	–	–	–
<i>o</i> = DGO*100/Stylet	19.4±2.0 (17.1–22.9)	–	–	–
Distance from dorsal gland orifice to stylet base	5.2±0.53 (4.6–6.0)	4±0.5 (3–4)	3.8±0.7 (3.2–4.4)	3.8±0.6 (3–4)
Anterior end to secretory-excretory pore	116±5.2 (110–124)	84±3.4 (79–89)	176.4±14.2 (156.2–189.6)	23±7.8 (16–38)
Anterior end to nerve ring	84±1.5 (82–86)	71±3.4 (66–77)	126.9±12.0 (110.3–138.6)	–

Continued

Anterior end to end of pharyngeal gland	127±4 (120–134)	139±15.5 (117–173)	239.7±2.2 (237.5–241.9)	–
Pharyngeal gland overlapping	21.9±3.9 (15–26.9)	–	–	–
Anterior genital tract	178.9±16.6 (168–203)	–	–	–
Posterior genital tract	173±5.7 (168–181)	–	–	–
Diam. at mid-body (MBD)	27.1±2.4 (23.8–31)	15±1.5 (13–18)	38.9±2.5 (35.9–41.0)	327±55.0 (259–430)
Diam. at anus (ABD)	17±0.89 (16.2–18.3)	10±0.6 (9–11)	71.7±101.3 (18.9–223.7)	–
Tail length	11.2±1.2 (9.8–13.3)	50±2.5 (46–53)	10.7±3.8 (5.0–13.2)	–
Hyaline length	4.2±0.51 (3.7–5.0)	15±2.1 (13–20)	–	–
Spicule length (along arc)	–	–	33.5±5.1 (28.4–40.3)	–
Maximum spicule width	–	–	4.1±0.6 (3.8–5.0)	–
Gubernaculum length	–	–	11.0±1.2 (10.1–12.6)	–
Number of tail annuli at ventral side	9.4±0.54 (9–10)	–	–	–
Scutellum diameter	3.4±0.28 (3.1–3.8)	–	–	–
Length of cervical region	–	–	–	243±37.9 (184–311)
Anterior end to end of metacarpus	–	–	–	81±10.5 (67–101)
Metacarpus diameter	–	–	–	36±6.4 (28–47)
Vulva slit length	–	–	–	18.7±2.9 (15–23)
Vulva width	–	–	–	23±3.2 (19–28)
Vulva-anus distance	–	–	–	16.2±2.2 (12–18)

in the botanical garden of Ghent University, with the accompanying Belgian weather conditions (cold winter seasons). This plant is an exotic plant that was imported from the Himalayas, which in its native habitat can be found growing at altitudes up to 5000 feet (about 1666m), and it is known to tolerate temperatures as low as 15 °F (–9°C). However, *M. incognita* is known as a tropical nematode and distributed in warmer climates (Wesemael et al., 2011). Most likely, this nematode originated from one of the tropical

plants that were imported and planted in the botanical garden of Ghent University. The fact that the growing area was covered by wood chips during winter time may have created more suitable conditions for *M. incognita*.

Meloidogyne incognita has already been reported in Belgium by Coolen et al. (1974) in nurseries growing multiflora tuberous begonias. However, without detailed taxonomical information, its species status can therefore not be assured.

Although in this study the presence of *S. brachyurus* and *M. incognita* in Belgium was demonstrated and a climate change-driven poleward migration of pests and plant pathogens is well known (Bebber, 2015; EPPO, 2019), we have insufficient data to state that the tropical nematodes found in this study form a potential threat nor that they are the result of global warming. Nevertheless, in the light of climate change it is highly important to gain more insights in tropical nematodes in more Northern countries. This current study provides the first report of *S. brachyurus* and confirms the presence of *M. incognita* in Belgium, although in a protected environment.

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References

- Andrássy, I. 1958. *Hoplolaimus tylenchiformis* Daday, 1905 (Syn. *H. coronatus* Cobb, 1923) and the genera of the subfamily Hoplolaiminae Filipjev, 1936. *Nematologica* 3:44–56.
- Bebber, D. P. 2015. Range-expanding pests and pathogens in a warming world. *Annual review of phytopathology* 53:335–56.
- Bebber, D. P., Ramotowski, M. A. and Gurr, S. J. 2013. Crop pests and pathogens move polewards in a warming world. *Nature Climate Change* 3:985–8.
- CABI. 2019. *Invasive Species Compendium*, International, C, Wallingford, UK, In: (ed.).
- Chitwood, B. G. 1949. Root-knot nematodes, part I. A revision of the genus *Meloidogyne* Goeldi, 1887. *Proceedings of the Helminthological Society of Washington* 16:90–104.
- Coolen, W. A., Bosmans, P. and Hendrickx, G. J. 1974. Een nematologisch en fytopathologisch survey-onderzoek in de multiflorenteelt, Rijksstation voor nematologie en entomologie, Merelbeke, Belgium.
- EPPO. 2019. EPPO global database. (available online at): <https://gd.eppo.int>
- Hartman, K. M. and Sasser, J. N. 1985. Identification of *Meloidogyne* species on the basis of differential hosts test and perineal pattern morphology. in Barker, K. R., Carter, C. C. and Sasser, J. N. (ed.), *Advanced Treatise on Meloidogyne*, North Carolina State University Graphics, Raleigh: A cooperative publication of the Department of Plant Pathology and the United States Agency for International Development, North Carolina, 69–77.
- Hunt, D. J. and Handoo, Z. A. 2009. Taxonomy, identification and principal species. in Perry, R. N., Moens, M. and Starr, J. L. (ed.), *Root-Knot Nematodes*, CAB Int, Wallingford, UK, 55–97.
- Janssen, T., Karssen, G., Verhaeven, M., Coyne, D. and Bert, W. 2016. Mitochondrial coding genome analysis of tropical root-knot nematodes (*Meloidogyne*) supports haplotype based diagnostics and reveals evidence of recent reticulate evolution. *Scientific Reports* 6:1–13.
- Kofoed, C. A. and White, A. W. 1919. A new nematode infection of man. *Journal of the American medical Association* 72:567–9.
- Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. 2012. NIH image to ImageJ: 25 years of image analysis. *Nature Methods* 9:671–5.
- Singh, P. R., Nyiragatare, A., Janssen, T., Couvreur, M., Decraemer, W. and Bert, W. 2018. Morphological and molecular characterisation of *Pratylenchus rwndae* n. sp. (Tylenchida: Pratylenchidae) associated with maize in Rwanda. *Nematology* 20:781–94.
- Steiner, G. 1938. Nematodes infesting red spiderlilies. *Journal of Agricultural Research* 56:1–8.
- Van Den Berg, E., Tiedt, L. R., Coyne, D. L., Ploeg, A. T., Navas-Cortés, J. A., Roberts, P. A., Yeates, G. W. and Subbotin, S. A. 2013. Morphological and molecular characterisation and diagnostics of some species of *Scutellonema* Andrásy, 1958 (Tylenchida: Hoplolaimidae) with a molecular phylogeny of the genus. *Nematology* 15:719–45.
- Van Den Berg, E., Tiedt, L. R., Stanley, J. D., Inserra, R. N. and Subbotin, S. A. 2017. Characterisation of some *Scutellonema* species (Tylenchida: Hoplolaimidae) occurring in Botswana, South Africa, Costa Rica and the USA, with description of *S. clavicaudatum* sp. n. and a molecular phylogeny of the genus. *Nematology* 19:131–73.
- Wesemael, W. M. L., Viaene, N. and Moens, M. 2011. Root-knot nematodes (*Meloidogyne* spp.) in Europe. *Nematology* 13:3–16.
- Whitehead, A. G. and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annales of Applied Biology* 55:25–38.
- Whitehead, A. G. 1968. Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four new species. *The Transactions of the Zoological Society of London* 31:263–401.