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PLANT DISEASE NOTES

First report of potato cyst nematode *Globodera pallida* (Stone, 1973) infecting potato (*Solanum tuberosum* L.) in Kenya.

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The potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis* are key pests of potato, subject to strict quarantine regulations worldwide (EPPO, 2013a). Indigenous to South America, they have spread to numerous potato-growing regions around the world. *G. rostochiensis,* was reported from Kenya in 2015 (Mwangi et al. 2015). During a nationwide survey conducted in 2016, *G. pallida* was detected in Kenya at an altitude of 2349 m above sea level in Nyandarua County (GPS "0.3150195, 36.48328"). Cysts were extracted from a 200 cm^3 soil sample following EPPO diagnostic protocol (EPPO, 2013a), and then handpicked under a stereo microscope. The PCN recovered showed morphometric characteristics of *G. pallida* and is reported here. For further studies, the Nyandarua field was re-sampled in February 2017, to collected additional soil samples and confirm the occurrence of *G. pallida*. From the collected cysts, 10 cysts were inoculated on potato (*Solanum tuberosum*) 'Shangi' in 5 pots with sterile soil and sand (1:1) and grown in a screenhouse for 3 months from May- July 2017; the multiplication rate at harvest was $\bar{\chi}$ = 3.6 and PCN were recovered from potato roots and soil. Morphometric characters showed: Granek's ratio (n = 33) ranged from 1.53 – 4.52 µm, ($\bar{\chi}$ = 2.78

± 0.78 µm), and the distance from anus –vulval basin was 34.03 – 91.45 µm ($\bar{\chi}$ = 52.75 ± 13.73 µm). The stylet length of the second-stage juveniles (J2s) (n = 97) ranged from 15.87 – 25.18 µm ($\bar{\chi}$ = 21.87 ± 1.43), stylet knobs displayed robust tulip/anchored-shape. The lengths of the hyaline tail (HT) and the true tail (TT) ranged from 15.54 – 50.44 µm ($\bar{\chi}$ = 23.94 ± 4.23) and 31.02 – 79.59 µm ($\bar{\chi}$ = 50.64 ± 5.71 µm), respectively. Body length (n = 40) fluctuated from 338.41 – 468.34 µm ($\bar{\chi}$ = 432.23 ± 24.95). DNA amplification was performed from 14 cysts and 25 J2s using the multiplex-PCR method adapted from Bulman & Marshall (1997) and the ITS1–5.8S-ITS2 regions (Tirchi et al. 2016). PCR cycling-parameters were adjusted to a 5-min initial denaturation phase and 37 PCR-cycles for multiplex-PCR (EPPO, 2013b). The species-specific primers ITS5/PITSp4 for *G. pallida* (265 bp) and AB28/TW81 primers (1188 bp) were used to amplify the small sub-unit of the 18s ribosomal RNA and the ITS region, respectively; PCR-amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, USA) and the DNA sequences were manually edited using BioEdit Sequence Alignment Editor; *in silico* analyses were conducted with the NCBI-BLAST tool. The Kenyan ITS5/PITSp4 sequences (NCBI accession no. MG309873) presented 100% similarity to the *G. pallida* isolates KJ409623.1 and AF016869 (Score = 481; E value = $5.02e^{-132}$), while the Kenyan AB28/TW81 sequence (NCBI accession no. MG309920) showed 95 and 94% similarity to the *G. pallida* isolates HF583248.1 and HQ670272.1 (Score = 1218 and 1221; E value = 0), respectively.

This first report of *G. pallida* in sub-Saharan Africa has paramount phytosanitary and regulatory implications for potato growers and traders, national extension services and policy makers in Kenya and the surrounding region.

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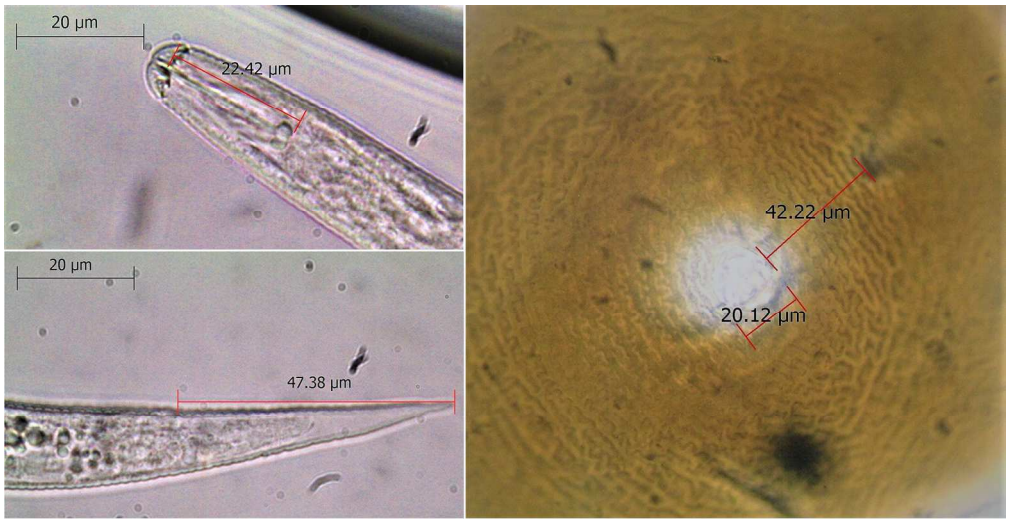

Figure 1. Morphometric analyses of G. pallida isolate from Kenya. Left up: stylet of a second-stage juvenile (J2) showing a characteristic rounded knob, measuring 22.42 µm from the base of the stylet up to the tip; left down: true tail of a J2, measuring 47.38 µm from the annuls up to the end of the tail/body; right: vulval cone of a female showing the measurement of the anus' length (20.12 µm) and the distance from the anus to the vulva of a female (42.22 µm) for the calculation of the Granek's ratio.

560x286mm (96 x 96 DPI)

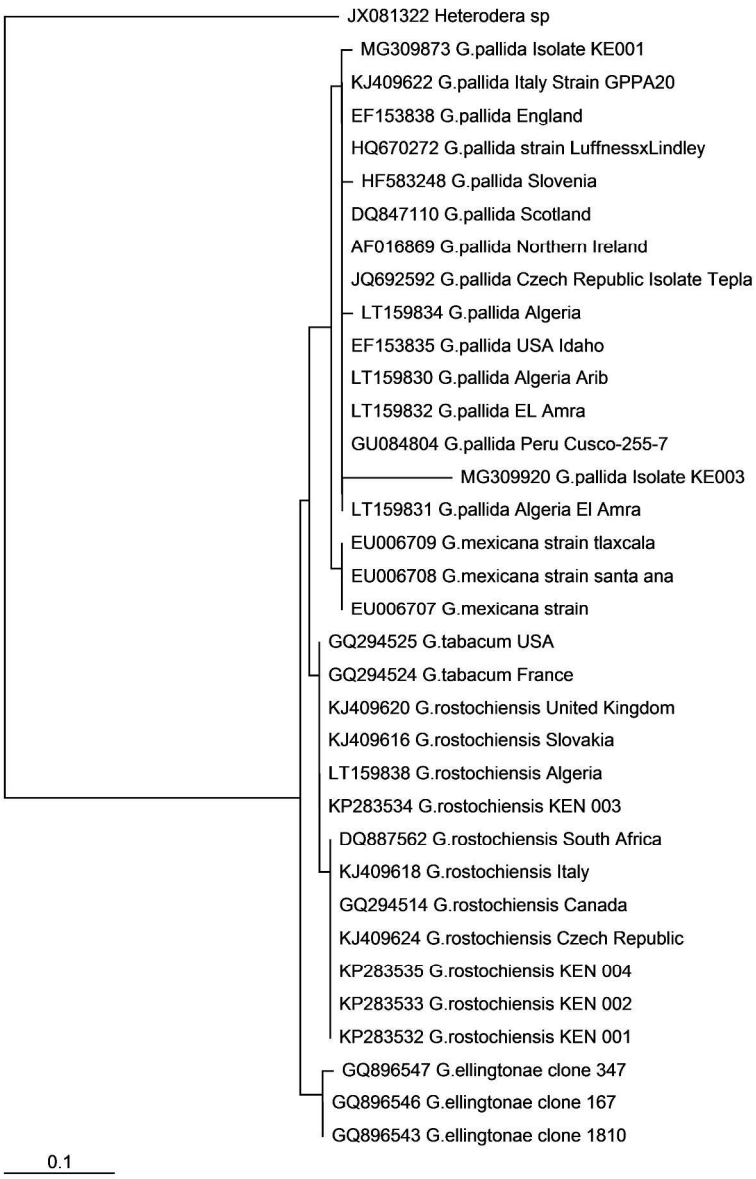

Figure 2. Phylogenetic tree showing the G. pallida isolates MG309920 and MG309873 (in blue) aligned with several G. pallida and G. rostochiensis isolates from Africa (Algeria, Tunisia South Africa), Europe and USA and G.ellingtonae, G. tabacum and G. mexicana. Phylogenetic tree was done using Tree Figure Drawing Tool Version 1.4.3 to Edit the Tree. 2006-2016, Andrew Rambaut Institute of Evolutionary Biology, University of Edinburgh.

692x966mm (144 x 144 DPI)

link to sequence MG309873:

https://www.ncbi.nlm.nih.gov/nuccore/MG309873.1?report=GenBank

Link to sequence MG309920

https://www.ncbi.nlm.nih.gov/nuccore/MG309920.1?report=GenBank