

## 1 PLANT DISEASE NOTES

2 **First report of potato cyst nematode, *Globodera rostochiensis* (Wollenweber, 1923), infecting potato**  
3 **(*Solanum tuberosum* L.) in Uganda.**

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23 Potato cyst nematodes (PCN; *Globodera rostochiensis* and *G. pallida*) are quarantine pests (EPPO  
24 2013) and have recently been reported from Kenya (Mburu et al. 2018) and Rwanda (Niragire et  
25 al. 2019). In East Africa, potato is an important staple food crop for millions of people, although  
26 current yields (10 t/ha) are far below potential (40 t/ha). A survey was conducted in Uganda to  
27 assess the incidence of PCN in farmers' potato fields. Soil samples were collected from 124 fields  
28 in areas neighboring Kenya and Rwanda (November 2018 - April 2019). Within each field a bulk  
29 sample of 2 kg using 20 cores was collected following a zigzag transects. Soil was thoroughly  
30 mixed, air-dried and sieved (1 mm mesh). Nematode cysts were extracted by taking three sub-  
31 samples of 200 cm<sup>3</sup> per field, using a Fenwick can (EPPO, 2013). Cysts were found in 17 fields  
32 (13.7%) with mean cyst counts of 2.6 cyst/ 200 cm<sup>3</sup> soil. One cyst each from two randomly selected  
33 fields; MBL 03 and MBL 07 (Table 1) were dissected under a stereo-microscope and 20 eggs/cyst  
34 were inoculated separately onto 3 potato plants of cv. 'Shangi' grown in 1 kg pots containing steam  
35 sterilized loam soil. Plants were maintained in the greenhouse and harvested after three months.  
36 Using a Fenwick can, a mean of 12 cyst per pot ( $\bar{x}$  = 83 eggs/ cyst) were extracted, of which 15

37 females and 31 second-stage juveniles (J2s) were used for morphometric analyses. Female length  
38 (L) ranged from 280.5 – 446.3  $\mu\text{m}$  ( $\bar{x}$  = 365.1  $\pm$  45.0  $\mu\text{m}$ ), width (W) from 200.3 – 440.5  $\mu\text{m}$  ( $\bar{x}$  =  
39 319.3  $\pm$  63.4  $\mu\text{m}$ ) and the L/W ratio was 1.2  $\pm$  0.2; Granek's ratio ( $n$  = 7) varied from 1.57 – 3.52  
40  $\mu\text{m}$ , ( $\bar{x}$  = 2.7  $\pm$  0.6  $\mu\text{m}$ ); the distance from anus to vulval basin was 26.31 – 62.73  $\mu\text{m}$  ( $\bar{x}$  = 50.1  
41  $\pm$  11.7  $\mu\text{m}$ ). The J2 stylet length ranged from 14.93 – 22.59  $\mu\text{m}$  ( $\bar{x}$  = 19.37  $\pm$  1.86) with round-  
42 shape stylet knobs. Length of the hyaline tail was 12.64 – 27.63  $\mu\text{m}$  ( $\bar{x}$  = 23.05  $\pm$  3.80) and the  
43 true tail ranged from 30.06 – 54.48  $\mu\text{m}$  ( $\bar{x}$  = 43.33  $\pm$  4.87  $\mu\text{m}$ ); body length of J2 varied from  
44 332.02 – 427.29  $\mu\text{m}$  ( $\bar{x}$  = 394.00  $\pm$  19.30); all morphometric parameters matched those described  
45 for *G. rostochiensis* (Subbotin & Franco, 2012). DNA was extracted (Qiagen DNeasy<sup>®</sup> Blood &  
46 Tissue kit; Qiagen Group; USA) and amplified using candidate ITSF/R primers targeting the ITS1-  
47 5.8S-ITS2 regions (modified from Mburu et al. 2018). One PCR reaction contained 0.5  $\mu\text{M}$  of  
48 each primer (forward and reverse), 5  $\mu\text{l}$  of 5X GoTaq<sup>®</sup> Buffer (Promega), 2 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$   
49 dNTPs, 0.125  $\mu\text{l}$  GoTaq<sup>®</sup> DNA polymerase (5 u/  $\mu\text{l}$ ), 1  $\mu\text{l}$  DNA template (final volume = 25  $\mu\text{l}$ ).  
50 PCR cycling included 2 min initial denaturation phase and 40 PCR-cycles. The PCR amplicons  
51 (500 bp) were sequenced and edited using BioEdit Sequence Alignment Editor. DNA sequences  
52 were analyzed using the NCBI-BLAST tool: sequences MN450308 and MN450309 showed  
53 similarity to the 5.8S ( $E$ = 4e<sup>-140</sup>; 97.64% identity) and 18S ( $E$ = 5e<sup>-139</sup>; 98.61% identity) ribosomal  
54 RNA gene of non-African *G. rostochiensis* isolates. A phylogenetic analysis showed that Ugandan  
55 populations cluster with Kenyan *G. rostochiensis* isolates but are less closely related to Rwandan  
56 populations or other *Globodera* species. Our findings highlight the need to conduct a  
57 comprehensive epidemiologic survey for developing a regional PCN-management strategy.

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61 Niragire, I., et al. 2019. Plant Dis. First Look. e-ISSN: 1943-7692

62 Subbotin, S., and Franco, J. 2012. Pages 337-353 in: Practical Plant Nematology. ISBN 978-607-  
63 715-078-7

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Sample ID	District	Regions	Altitude (m.a.s.l)	GPS North	GPS East	Generalized wilting of potato crop	Patchiness observed in the crop	Stunted growth observed in potato crop	Potato variety planted	Cysts per 200 cm <sup>3</sup> soil
KPR 04	Kapchorwa	North East	2048	01°20.304'	034°24.519'	Yes	Yes	Yes	Rwangume	1
KPR 19	Kapchorwa	North East	2113	01°04.290'	034°15.997'	Yes	Yes	No	Unknown	1
KPR 26	Kapchorwa	North East	2072	01°22.865'	034°28.038'	No crop	No crop	No crop	N/A	1
KWN 51	Kween	North East	2222	01°22.180'	034°26.996'	No crop	No crop	No crop	N/A	1
MBL 03	Mbale	North East	1966	01°02.128'	034°14.307'	Yes	No	No	Rwangume	4
MBL 04	Mbale	North East	1894	01°02.395'	034°14.810'	Yes	Yes	Yes	Rwangume	6
MBL 07	Mbale	North East	2037	01°04.565'	034°15.652'	Yes	Yes	No	Rwangume	5
MBL 10	Mbale	North East	1929	01°04.415'	034°14.719'	No	Yes	Yes	Rwangume	4
MBL 13	Mbale	North East	1952	01°03.780'	034°15.322'	Yes	No	Yes	Rwangume	2
MBL 62	Mbale	North East	2078	01°21.981'	034°28.841'	No	No	No	Rwangume	2
KBL 28	Kabale	South West	2332	01°23.130'	029°56.908'	No crop	No crop	No crop	N/A	1
KBL 33	Kabale	South West	2241	01°26.646'	029°57.864'	No crop	No crop	No crop	N/A	1
KSR 01	Kisoro	South West	2028	01°19.226'	029°40.556'	No	Yes	Yes	Kinigi	2
KSR 11	Kisoro	South West	2246	01°12.260'	029°44.490'	No	Yes	Yes	Kinigi	1
KSR 03	Kisoro	South West	2219	01°20.692'	029°40.835'	No	Yes	Yes	Rangume	10
KSR 08	Kisoro	South West	1996	01°18.912'	029°43.111'	No crop	No crop	No crop	N/A	1
RBD 20	Rubanda	South West	2019	01°16.101'	029°51.450'	No crop	No crop	No crop	N/A	2

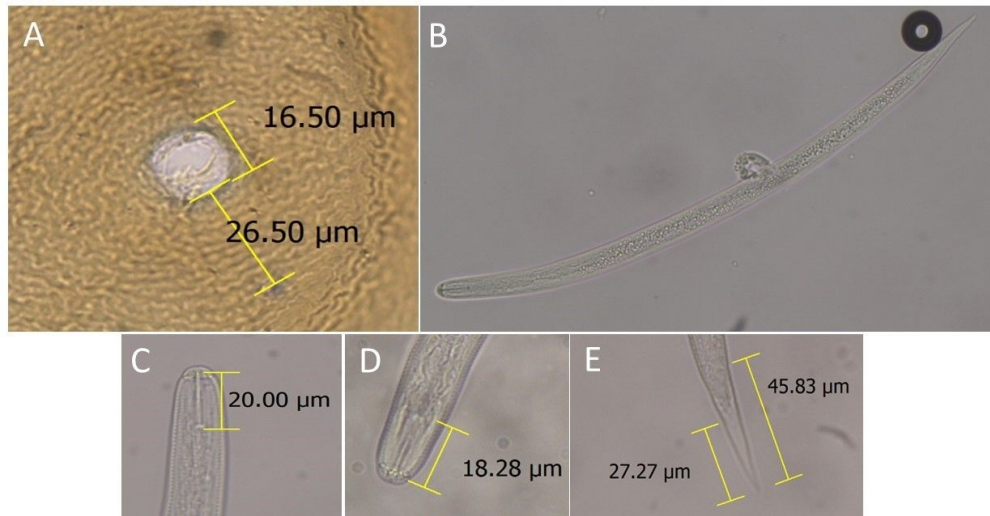


Figure 1. Morphometric analysis of *Globodera rostochiensis* isolate from Uganda. (A) females' vulval cones showing the measurement of the vulva diameter (16.50  $\mu\text{m}$ ) and the distance from the vulva to the anus (26.50  $\mu\text{m}$ ); (B) Body habitus of a J2; (C & D) Pharyngeal region of a second-stage juvenile (J2), presenting stylet measurements from the base of the stylet to the tip and displaying rounded stylet-knobs (D); (E) J2's true tail (47.82  $\mu\text{m}$ ) and hyaline tail (25.07 $\mu\text{m}$ ).

261x135mm (120 x 120 DPI)

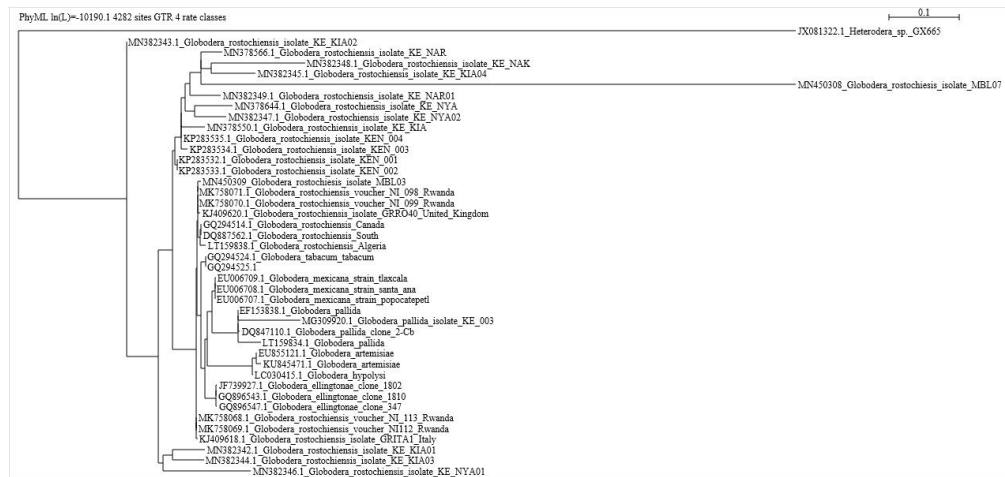


Figure 2. Phylogenetic tree presenting the *Globodera rostochiensis* isolates MN450308 and MN450309 aligned with isolates of seven *Globodera* spp., including isolates from Kenya and Rwanda. Phylogenetic tree was done using Tree Figure Drawing Tool Version 1.4.3 to Edit the Tree. 2006-2016, Andrew Rambaut Institute of Evolutionary Biology, University of Edinburgh.

223x105mm (144 x 144 DPI)