

Description of *Globodera ellingtonae* n. sp. (Nematoda: Heteroderidae) from Oregon

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Abstract: A new species of cyst nematode, *Globodera ellingtonae*, is described from soil collected from a field in Oregon. Second-stage juveniles (J2) of the species are characterized by body length of 365–515 μm , stylet length of 19–22.5 μm , basal knobs rounded posteriorly and pointed anteriorly, tail 39–55 μm , hyaline tail terminus 20–32.5 μm , and tail tapering uniformly but abruptly narrowing and constricted near the posterior third of the hyaline portion, ending with a peg-like, finely rounded to pointed terminus. Cysts are spherical to sub-spherical, dark to light brown and circumfenestrate and cyst wall pattern is ridge-like with heavy punctations. Males have a stylet length of 21–25 μm and spicule length of 30–37 μm with a pointed thorn-like tip. Females have a stylet length of 20–22.5 μm , one head annule and labial disc, heavy punctations on the cuticle, and short vulval slit 7.5–8 μm long. Morphologically this new, round-cyst species differs from the related species *G. pallida*, *G. rostochiensis*, *G. tabacum* complex and *G. mexicana* by its distinctive J2 tail, and by one or another of the following: shorter mean stylet length in J2, females and males; number of refractive bodies in the hyaline tail terminus of J2; cyst morphology including Granek's ratio; number of cuticular ridges between the anus and vulva; and in the shape and length of spicules in males. Its relationship to these closely related species are discussed. Based upon analysis of ribosomal internal transcribed spacer (ITS) sequences, *G. ellingtonae* n. sp. is distinct from *G. pallida*, *G. rostochiensis*, *G. tabacum* and *G. mexicana*. Bayesian and Maximum Parsimony analysis of cloned ITS rRNA gene sequences indicated three clades, with intraspecific variability as high as 2.8%. *In silico* analysis revealed ITS restriction fragment length polymorphisms for enzymes Bsh 1236I, Hinf I, and Rsa I that overlap patterns for other *Globodera* species.

Key words: *Globodera ellingtonae*, detection, diagnosis, morphology, new species, potato, RFLP, *Solanum tuberosum*, taxonomy.

The taxonomy of *Globodera* has been advanced by numerous papers (Golden and Ellington, 1972; Miller and Gray, 1972; Mulvey, 1973; Stone, 1973; Miller, 1983; Mulvey and Golden, 1983; Stone, 1983; Sturhan, 1983; Wouts, 1984; Golden, 1986; Baldwin and Mundo-Ocampo, 1991; Mota and Eisenback, 1993a, 1993b, 1993c; Siddiqi, 2000; den Nijs and Karssen, 2004), and molecular diagnostic and/or phylogenetic studies (Baldwin and Schouest, 1990; Ferris et al., 1995; Szalanski et al., 1997; Thomas et al., 1997; Fleming and Powers, 1998; Wouts and Baldwin, 1998; Subbotin et al., 2000; Manduric et al., 2004; Madani et al., 2005; Skantar et al., 2007; Quader et al., 2008; Subbotin et al., 2010a, 2010b; Skantar et al., 2011). The most important members of the genus are the potato cyst nematodes (PCN, here in meaning *Globodera* species pathogenic on potato), which represent a significant threat to potato worldwide and to the U.S. potato industry, which produces annual crops valued at nearly \$3.45 billion (Agricultural Statistics Board, 2011). In the United States, the first PCN species detected was the golden nematode, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959, which was identified in New York in 1932 and has remained confined there as a result of regulatory and management practices (Brodie, 1998). More recently, the pale potato cyst nematode, *G. pallida* (Stone, 1973) Behrens, 1975, was discovered in Idaho (Hafez et al.,

2007). Since then, an extensive survey of the U.S. potato growing acreage has been conducted jointly by the various state Departments of Agriculture and the USDA Animal and Plant Health Inspection Service to determine the extent of PCN distribution. Nematodes suspected of being a PCN are analyzed by morphological and molecular means at the USDA Nematology Laboratory in Beltsville, MD.

In 2008, three nematode samples distinct from the known PCN species were received at Beltsville. These atypical cyst nematodes had been isolated from an Oregon field in which potato and other crops had been grown, and from two agricultural fields within Idaho. In preliminary reports, this unnamed nematode was referred to as a new *Globodera* species (Fraleley et al., 2009) or as atypical *Globodera* populations from Oregon and Idaho (Skantar et al., 2011). Due to the limited quantity of sample material then available, a full description of all major developmental stages was not possible at that time. Additional field sampling at the Oregon location and subsequent propagation of the nematode have permitted extensive morphometric characterization of second-stage juveniles (J2), females, males, and cysts and additional molecular characterization. These observations indicate that the Oregon isolate represents a new species, described here as *Globodera ellingtonae*. Morphological characteristics that define the species are described, as are features that overlap with other *Globodera* species. Intraspecific variation present within the ribosomal internal transcribed spacer (ITS) rRNA region is also described.

MATERIALS AND METHODS

Cysts were collected during PCN surveys in May 2008 from a Powell Butte, Oregon, field with a cropping

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history of potato and other crops, and in August and September 2008 from two agricultural fields (#167, 347) in Idaho with unknown cropping histories. The Idaho isolate #167 cysts came from a field in Caribou County and the Idaho isolate #347 cysts were from a field in Teton County. Additional specimens used to prepare the description were obtained from greenhouse cultures on *Solanum tuberosum* established by Inga Zasada and Russell Ingham (USDA-ARS and Oregon State University, respectively, Corvallis, OR) from nematodes they collected at the Powell Butte site in 2009. Juveniles were hatched from cysts that had been sieved from fresh soil and kept in water in Syracuse watch glasses in the laboratory. Males, females and J2 were fixed in either warm (40°C) or hot (80°C) 4% formalin, processed to glycerin with a rapid method (Seinhorst, 1959), and mounted in anhydrous glycerin on microscope slides (Hooper, 1970; Golden, 1990). Cysts were removed from soil samples by sieving, fixed for 12 hr in 3% formaldehyde and similarly processed to glycerin. Photomicrographs of cyst vulval cones, females, males and J2 were taken with a Q-Imaging Retiga EXi Color Digital Camera (Q-Imaging, Austin, TX) attached to a Leica Wild MPS48 Leitz DMRB compound microscope (Leica Microsystems, Wetzlar, Germany), and measurements were made with an ocular micrometer on the same microscope. For head and tail variations, photomicrographs of live specimens were taken using an ocular micrometer on a Zeiss Ultraphot II with DIC (differential interference contrast) (Carl Zeiss, Jena, Germany) and a Q-I Micropublisher 5 color digital camera (Q-Imaging Inc., Austin, TX). Black and white tail profiles were created in Adobe Photoshop CS (Adobe Systems Inc., San Jose, CA, USA). Filter effects were applied to the images (Sketch → Photocopy: with low detail and high darkness levels) to create a sketch-like appearance. Brightness/contrast levels were modified to produce the background for the images, and brush tools were used to more clearly reveal stylets and lips. All original images are on file for comparison with the altered images.

Preparation of genomic DNA from juveniles, amplification of the ITS rRNA gene region, purification of amplicons, and cloning were carried out as described previously (Skantar et al., 2011). Amplification of the 28S rRNA region was as described in DeLey et al. (2005) using primers D2A and D3B (Ye et al., 2007). Sequencing was performed at the University of Maryland Center for Biosystems Research. Fourteen clones were sequenced from juveniles obtained in the initial sampling from Powell Butte (new GenBank accession numbers JF739926-JF39934 and previous sequences GQ896542, GQ896544, and GQ896545). Twenty-seven additional clones were sequenced from five morphologically variable juveniles from a single cyst (JF39899-JF39925). A total of 16 clones were sequenced from laboratory cultures of the Oregon population of *G. ellingtonae* n. sp. (JF739872-JF739887). Sequence obtained previously

representing four juveniles from the single available cyst of Idaho sample #167 was GQ896546. Eleven clones were sequenced from Idaho sample #347 (new, JF739888-JF739898; previous, GQ896547). New 28S rDNA D2-D3 expansion segment sequences include accession numbers JN712217-JN712224.

DNA sequences were assembled with Sequencher 5.0 (Genecodes, Ann Arbor, MI). The program MUSCLE (Edgar, 2004) was used for DNA sequence alignments, with minor corrections made in GeneDoc (Nicholas et al., 1997). *Globodera rostochiensis* sequence FJ212167 was specified as the outgroup. JModelTest (Guindon and Gascuel, 2003; Posada, 2008; Posada and Crandall, 2001) was used to determine the model of DNA evolution that best fit the data. The Akaike Information Criterion (AIC) values from JModelTest were used to run the Bayesian Interference (BI) analysis with the program MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) under the model GTR + I + G, with a random seed, and four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 1,000,000 generations with subsampling frequency 1,000. The log-likelihood values stabilized after approximately 5,000 generations; sample points obtained prior to convergence were discarded as burn-in. Convergence was evaluated through the potential scale reduction factor (PSRF) and further confirmed through the use of AWTY, a web-based interface for graphical visualization of MCMC diagnostics (Nylander et al., 2008). Tree topologies were used to generate a 50% majority rule consensus tree in PAUP* version 4.0b10 (Swofford, 2003). Maximum Parsimony (MP) procedures employed a heuristic search, random taxon addition, MaxTrees = 500, and tree bisection-reconnection (TBR) branch-swapping. Gaps were treated as missing data. Support for each node was estimated by bootstrap searches with 1000 replicates. BI and MP trees were drawn with FigTree 1.3.1 (Rambaut, 2009) and CorelDraw X4 (Corel, Mountain View, CA).

RESULTS AND DISCUSSION

Globodera ellingtonae n. sp.
(Figs. 1-10) (Tables 1-4)

Oregon population: *Holotype* (female): Length (including neck) 545 µm; width 386 µm; L/W ratio 1.4; stylet 22.5 µm; dorsal esophageal gland orifice 5.0 µm from the stylet base; neck length 129 µm; neck width 75 µm.

Females (n = 10): Body white becoming yellow to pale brown as eggs mature, ovate to rounded or subspherical in shape with elongate, protruding neck, rounded posteriorly (Fig. 1A-D); dorsal esophageal gland orifice ≥ 5 µm distance from base of stylet; outer cuticular layer marked by rugose pattern and the inner layer by heavy punctations (Fig. 1E,F,H,I); subcrystalline layer absent. Head slightly set off from neck, bearing one prominent annule and labial disc. Cephalic framework

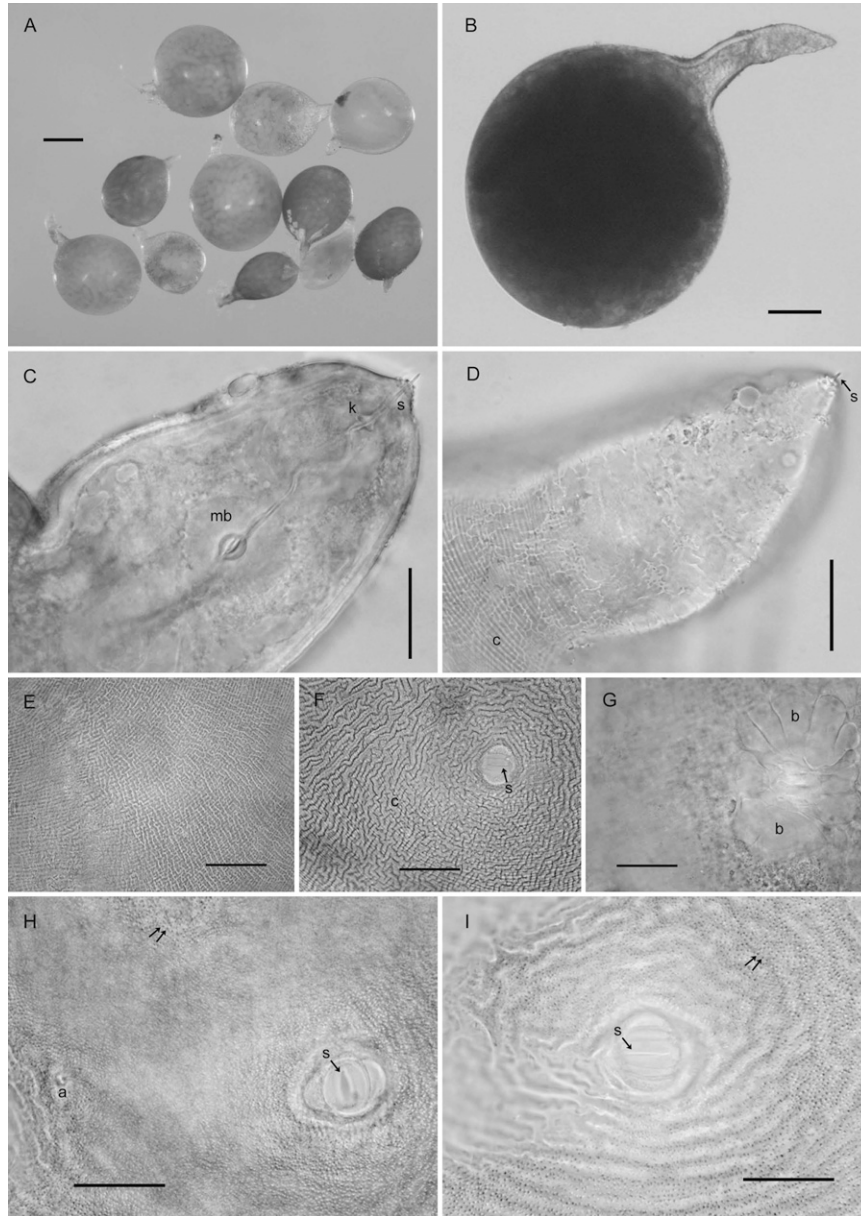


FIG. 1. Photomicrographs of females of *Globodera ellingtonae* n. sp. from Oregon: A, B, Whole females. C, Anterior region with s = stylet, k = knobs, mb = median bulb. D, Anterior region with s = stylet, c = cuticular ridges on neck. E, Cuticular pattern at mid body. F-I, Anal-vulval regions with arrows in F, H, I showing vulval slit (s) and c = cuticular ridges in F, double arrows = punctations on body wall in H, I, a = anal area in H, and b = clustered vulval denticles/bullae-like structures beneath vulval area in G. (Scale bars: A = 250 μ m; B = 100 μ m; C-I = 25 μ m).

weakly developed. Stylet with well-developed, posteriorly inclined knobs (Fig. 1C,D). Median bulb large, nearly spherical with well-developed valve (Fig. 1C). Esophageal glands mostly obscured, contained in a single lobe. Excretory pore about two annules posterior to hemizonid and located at or near base of neck. Vulva slit-like, small, measuring 7-8 μ m (Fig. 1F,H,I). Vulval bodies, highly variable in size, shape and generally clustered, usually discernible underneath the vulva (Fig. 1G). Anus prominent, often with clear circular area around it (Fig. 1H).

Cysts (n = 20): Light brown to brown in color, spherical to subspherical with a protruding neck (Fig. 2A); young cysts occasionally with clustered vulval bodies of variable

size and shape similar to bullae (Figs. 3C-D; 2J). Anus prominent and visible in all specimens, often with clear circular surrounding region (Figs. 3A,B,D; 2G-J). Subcrystalline layer absent. Vulval region intact or fenestrated with a single, circumfenestrate opening occupying all or part of vulval basin. Vulval bridge, underbridge and true bullae absent. Cyst wall pattern ridge-like to irregular, wavy or whorled (Figs. 3A,B,E,F), with heavy punctations (Fig. 2D-I).

Males (n = 20): Length highly variable (Table 2, Fig. 4A). Body slender, vermiform, tapering slightly at both extremities. Cuticle with distinct annulations. Lip region rounded to hemispherical, continuous or slightly

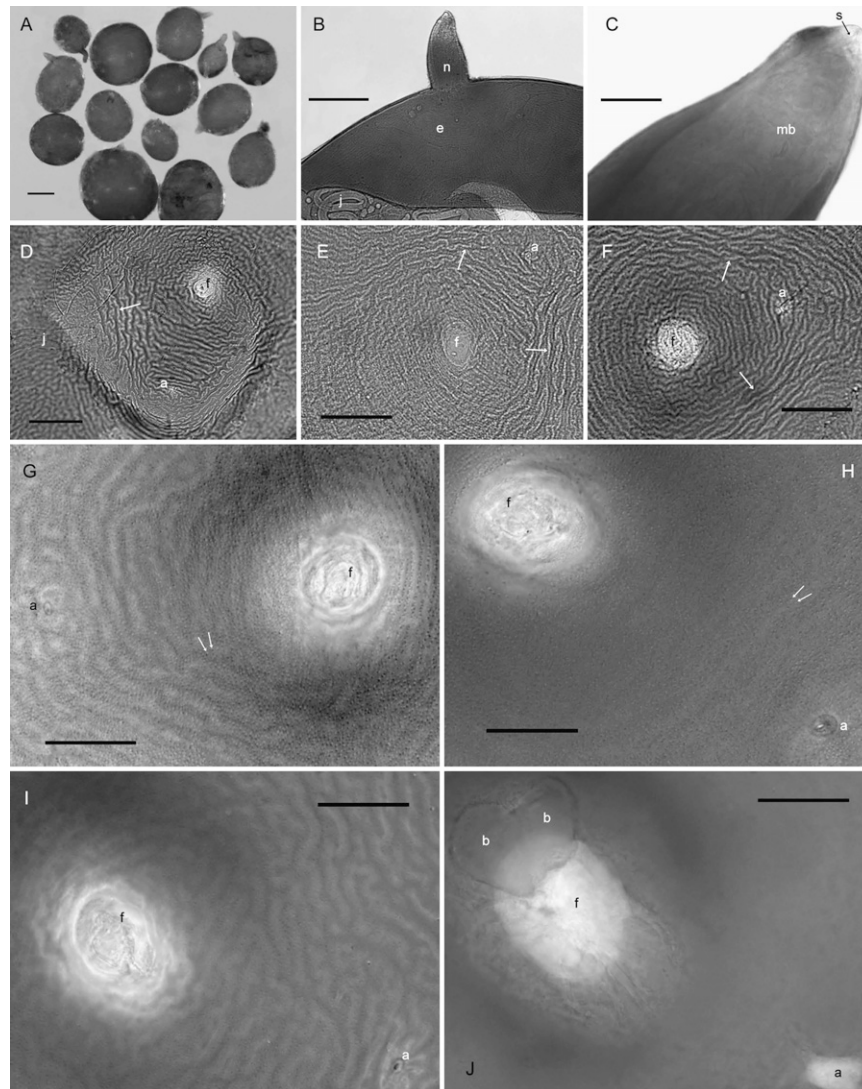


FIG. 2. Photomicrographs of whole, anterior, and terminal areas of cysts of *Globodera ellingtonae* n. sp. from Oregon: A, whole cysts. B, C, anterior region showing e = egg, j = juvenile, and n = neck in B, and s = stylet and mb = median bulb in C. D-J, Anal-vulval regions with f = vulval fenestra and a = anal area in D-J, j = juvenile, arrow = ridges on cyst wall in D-F; double arrows in G, H = punctations on cyst wall, and b = vulval bodies/bullae-like structures in J. (Scale bars: A = 250 μ m; B = 100 μ m; C, G-J = 25 μ m); D-F = 50 μ m).

set-off, with five indistinct annules (Fig. 4B-E). Cephalic framework heavily sclerotized (Fig. 4E). Lateral field with four incisures, outer bands with scattered, incomplete areolation (Fig. 4F). Tail tip rounded or bluntly conoid (Fig. 4G-I). Stylet well-developed with prominent rounded to posteriorly directed knobs (Fig. 4C-E), dorsal gland orifice near stylet base. Valved median bulb prominent (Fig. 4D). Isthmus and esophageal glands typical for the genus, but with obscure nuclei. Excretory pore two annules posterior to hemizonid, at anterior half of glands. Male gonad about 40-60% of body length. Spicules slightly curved, tips pointed, thorn-like (Fig. 4H,I), gubernaculum 8-15 μ m long (Fig. 4G).

Second-stage juveniles (n = 106): Body vermiform, tapering at both extremities, more so posteriorly (Fig. 5A). Some specimens bent at four points along the body when hatched or cracked open from eggs. Head

rounded, slightly set off with prominent cephalic sclerotization, bearing four to five annules (Figs. 5B-E; 6A-E). Stylet short, well developed; basal knobs rounded posteriorly, with a forward projection anteriorly (Fig. 5C), dorsal gland orifice distance from stylet base 7.5 μ m (6.0-8.5) (Fig. 6A,B). Excretory pore located near anterior end of esophageal glands. Phasmids not observed. Tail tapering uniformly but abruptly narrowing with a pronounced to slight constriction near the posterior third of the hyaline portion, ending with a peg-like, finely rounded to pointed terminus (Figs. 5F-J; 6G-M). Hyaline tail terminus often with up to 7 minute refractive bodies (Figs. 5F,H,J; 6H). Cuticular annulations distinct with more prominent annules near the tail terminus (Fig. 6I). Lateral field consisting of four incisures, with occasional areolation (Figs. 5F, 6F). In a few specimens, tail and anterior end with three incisures, with outer two

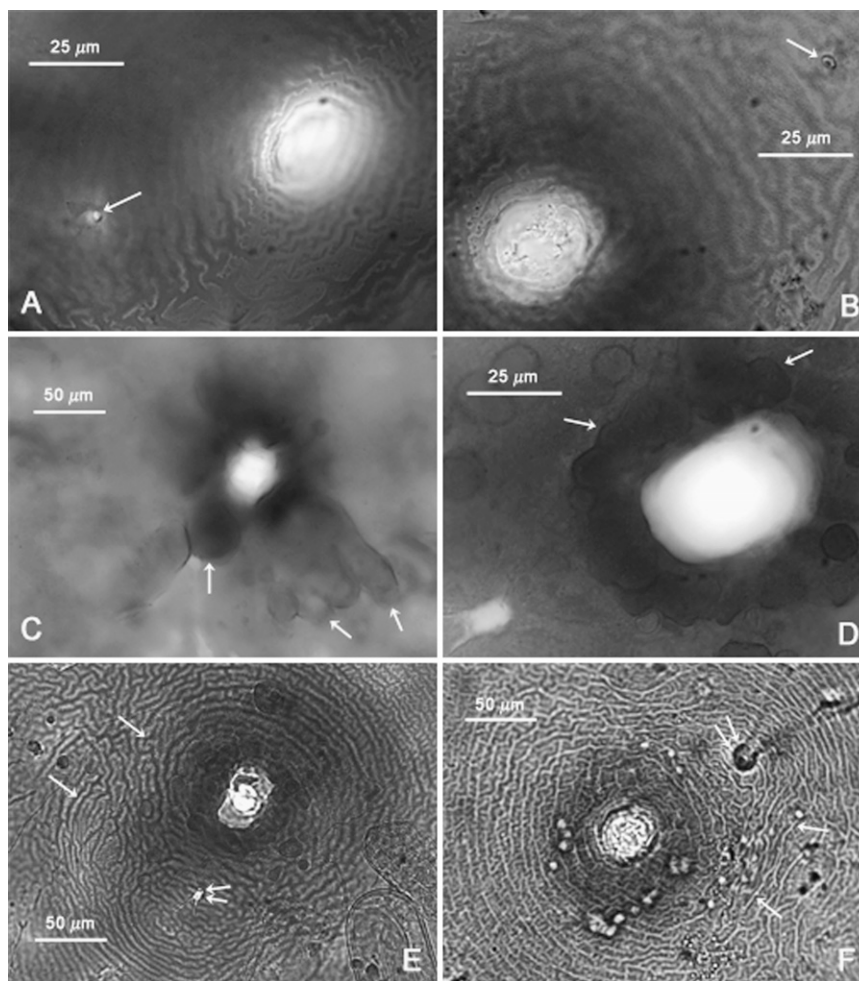


FIG. 3. Photomicrographs of terminal areas of cysts of *Globodera ellingtonae* n. sp. from Oregon: A-F, Anal-vulval regions with single arrows in A-B, C-D, and E-F showing anal areas, clustered vulval bodies/bullae-like structures, and cyst wall patterns, respectively, and the double arrows in E-F showing anal areas. (Modified from Skantar et al., *Phytopathology*, 2011).

showing areolation, and with four incisures at mid-body. Head and tail variations are shown in Fig. 7 with head showing rounded to slightly set off lip region, rounded to anteriorly directed stylet knobs, and tail terminus bluntly pointed to peg-like.

Type host and locality: The Oregon population was collected in May 2008 during PCN surveys from a Powell Butte, Oregon, field with a history of potato and other crops. Additional specimens were from greenhouse cultures on *Solanum tuberosum* ‘Mazama’ established by Inga Zasada and Russell Ingham in 2009 from nematodes collected from the Oregon site.

Type specimens: Specimens from aforementioned greenhouse cultures on *Solanum tuberosum* ‘Mazama’: Holotype (female) on slide T-653t, paratype (females) on slides T-6050p to T-6059p, paratype (cysts) on slides T-6060p to T-6075p and in vials T-568p and T-569p, paratype (males) on slides T-6076p to T-6087p and in vial T-572p, paratype (second-stage juvenile) on slides T-6088p to T-6091p and in vials T-568p to T-571p. Specimens isolated from Oregon type host and locality: paratypes (second-stage juveniles) on slides T-5847p to T-5862p

and in vials T-535p to T-541p, paratypes (cysts) on slides T-5863p to T-5870p, deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland. Additional paratype males, juveniles, and cysts deposited in the University of California-Riverside Nematode Collection, Riverside, California; the Rothamsted Nematode Collection, York, England; the University of Tennessee Nematode Collection, Knoxville, Tennessee; and the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada.

Idaho isolate #167, Cysts (n = 2): Light brown color, spherical with a protruding neck (Fig. 8A). Cyst wall pattern ridge-like with heavy punctations (Fig. 8B). Vulval region intact or fenestrated with a single circumfenestrated opening that occupies all or part of vulval basin (Fig. 8C,D). Whole fenestra length 22.5-25 μm; distance from nearest edge of fenestra to anus 40-52 μm; number of ridges between anus and nearest edge of fenestra 10-14; Granek’s ratio 1.8-2.1.

Second-stage juveniles (n = 10): Body vermiform, specimens were bent at four points along the body when hatched or cracked open from eggs. Tail tapering

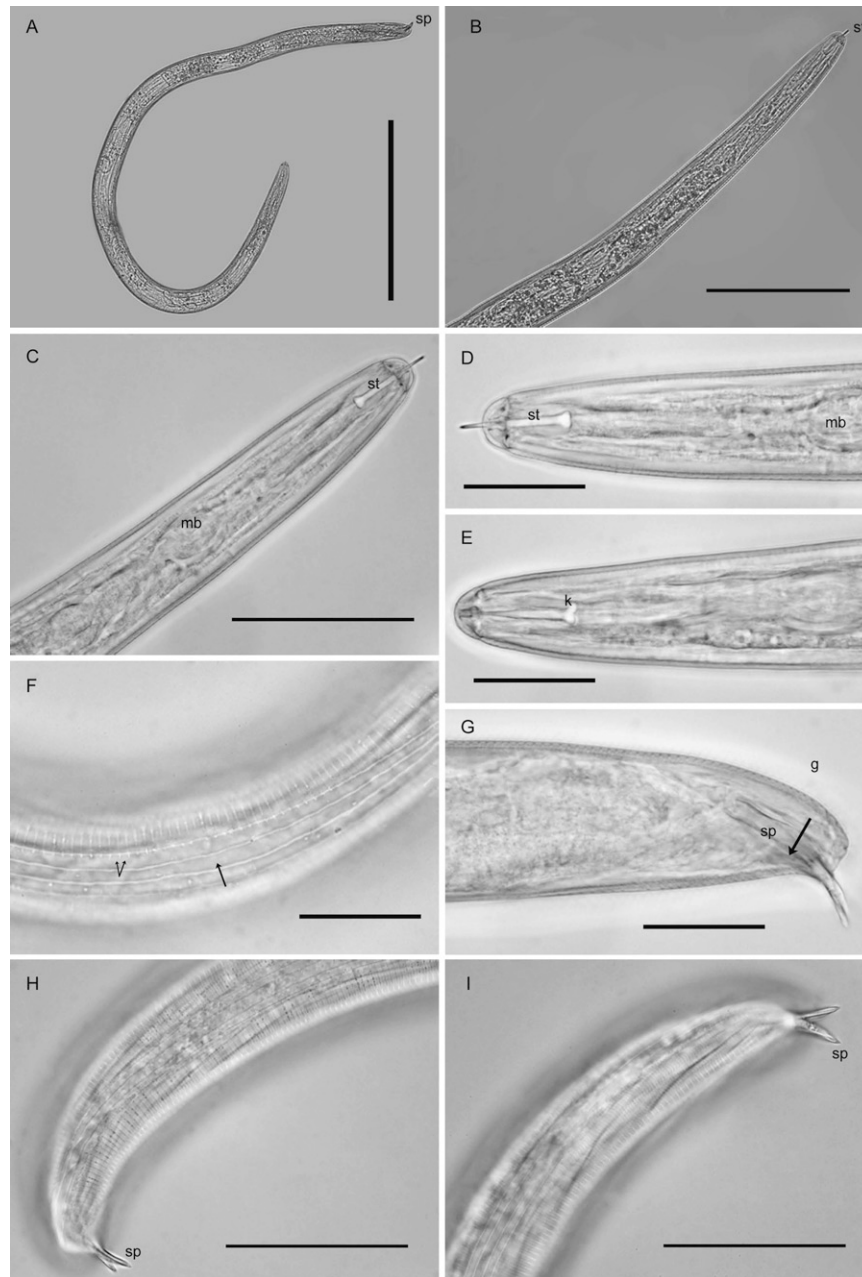


FIG. 4. Photomicrographs of males of *Globodera ellingtonae* n. sp. from Oregon: A, Whole male. B-E, Anterior regions showing stylet and part of esophagus, with st = stylet in C, D, mb = median bulb in C, and k = stylet knobs in E. F, Lateral field at mid-body, single arrow = lateral lines, double arrow = areolation. G-I, Posterior regions, showing spicules (sp) with pointed thorn-like tips and g = gubernacula in G. (Scale bars: A = 250 μ m; B = 100 μ m; C, H, I = 25 μ m).

uniformly and usually bluntly rounded to pointed at the terminus (Fig. 8I-M). Tail of one specimen similar to tail typical of the Oregon population, i.e., tail abruptly narrowing with a slight constriction near the posterior third of the hyaline portion, ending with a finely rounded to pointed terminus (Fig. 8I). Lateral field with four incisures. Head rounded, slightly set off with prominent cephalic sclerotization, bearing four annules. Stylet well-developed; basal knobs rounded and with a forward projection on anterior side. Excretory pore located near the anterior end of the esophageal

glands. Phasmids not observed. Length 425-485 μ m (451.7 ± 23.74); stylet 21-23 μ m (22.3 ± 0.52); tail 42-50 μ m (48 ± 3.09); hyaline tail terminus 20-30 μ m (24.6 ± 3.26).

Host and locality: Collected during PCN surveys of soil from an agricultural field with unknown cropping history in Caribou County, Idaho.

Specimens: Second-stage juveniles on slides T-5871p to T-5873p and in vial T-542p, cysts on slides T-5874p to T-5877p deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

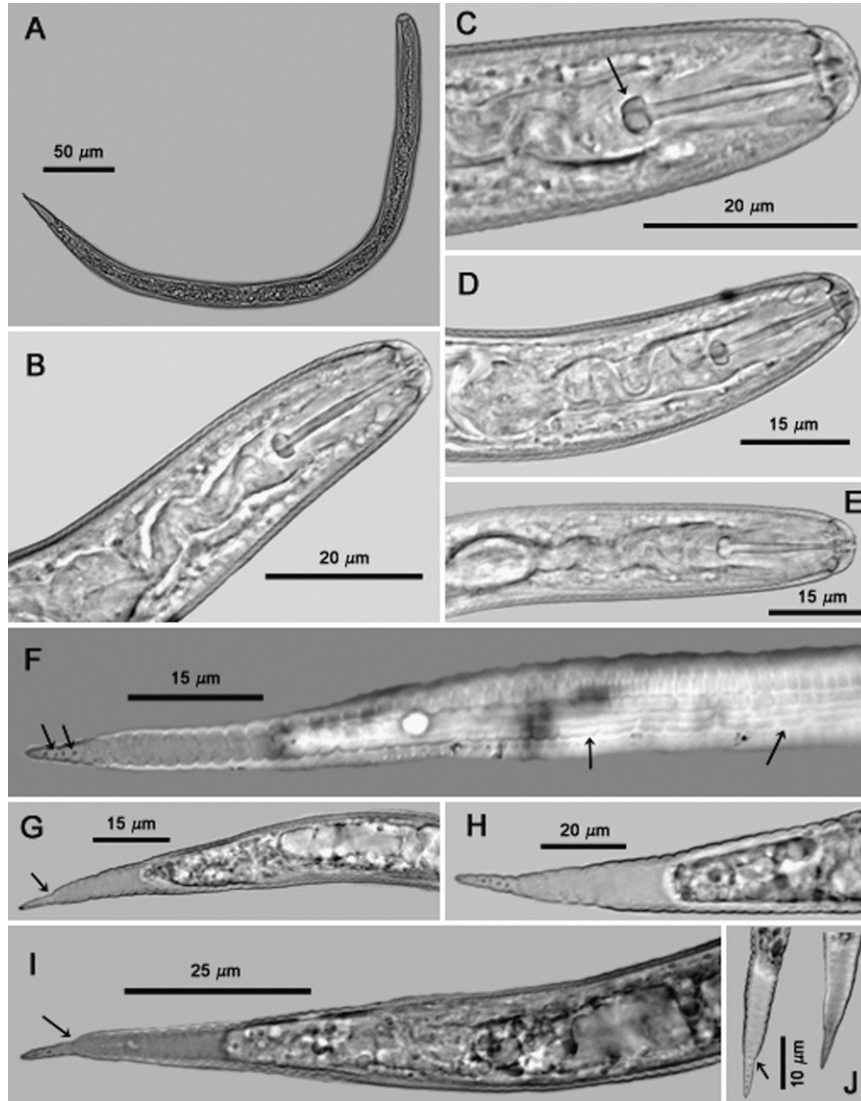


FIG. 5. Photomicrographs of second-stage juveniles of *Globodera ellingtonae* n. sp. from Oregon: A, Whole juvenile. B-E, Anterior regions showing stylet and part of esophagus, with C showing high stylet knobs (arrow). F, Tail showing areolated nature of lateral field with four lines (right arrows) and refractive bodies in tail ending with a peg-like finely rounded to pointed terminus (left arrows). G-J, tail variations, with G, I, J (arrows) showing abrupt narrowing of tail with pronounced to slight constriction. (Modified from Skantar et al., *Phytopathology*, 2011).

Idaho isolate #347, Cysts (n = 2): Light brown color, spherical with a protruding neck. Cyst wall pattern ridge-like with heavy punctations. Vulval region intact or fenestrated, with a single circumfenestrate opening occupying all part of vulval basin. Whole fenestra length 25 µm; distance from nearest edge of fenestra to anus 30-55 µm; number of ridges between anus and nearest edge of fenestra 8-14; Granek's ratio 1.2-2.2.

Second-stage juveniles (n = 8): Body vermiform, specimens were degraded and bent at four points along the body when hatched or cracked open from eggs. Tail tapering uniformly and usually bluntly rounded to pointed at the terminus. Lateral field with four incisures. Head rounded, slightly set off with prominent cephalic sclerotization, bearing four annules. Stylet well-developed; basal knobs rounded and with a forward projection on anterior side (Fig. 8F). Length 421-435 µm (428 ± 9.89);

stylet 22-23 µm (22.5 ± 0.32); tail 48 µm; hyaline tail terminus 22.5-30 µm (25.7 ± 2.52).

Host and locality: Collected during PCN surveys of soil from an agricultural field with unknown cropping history in Teton County, Idaho.

Specimens: Cysts on slides T-5878p and T-5879p, second-stage juveniles in vial T-543p deposited in the U.S. Department of Agriculture Nematode Collection, Beltsville, Maryland.

Diagnosis and Relationships: *Globodera ellingtonae* n. sp. is characterized by J2 with body length of 450 µm (365-515); stylet length 20.9 µm (19-22.5) with rounded to high basal knobs with a forward projection on anterior side; areolated lateral field with 4 incisures; tail 46.7 µm (39-55) long, tapering uniformly but abruptly narrowing, with a pronounced to slight constriction near the posterior third of the hyaline portion, ending with a

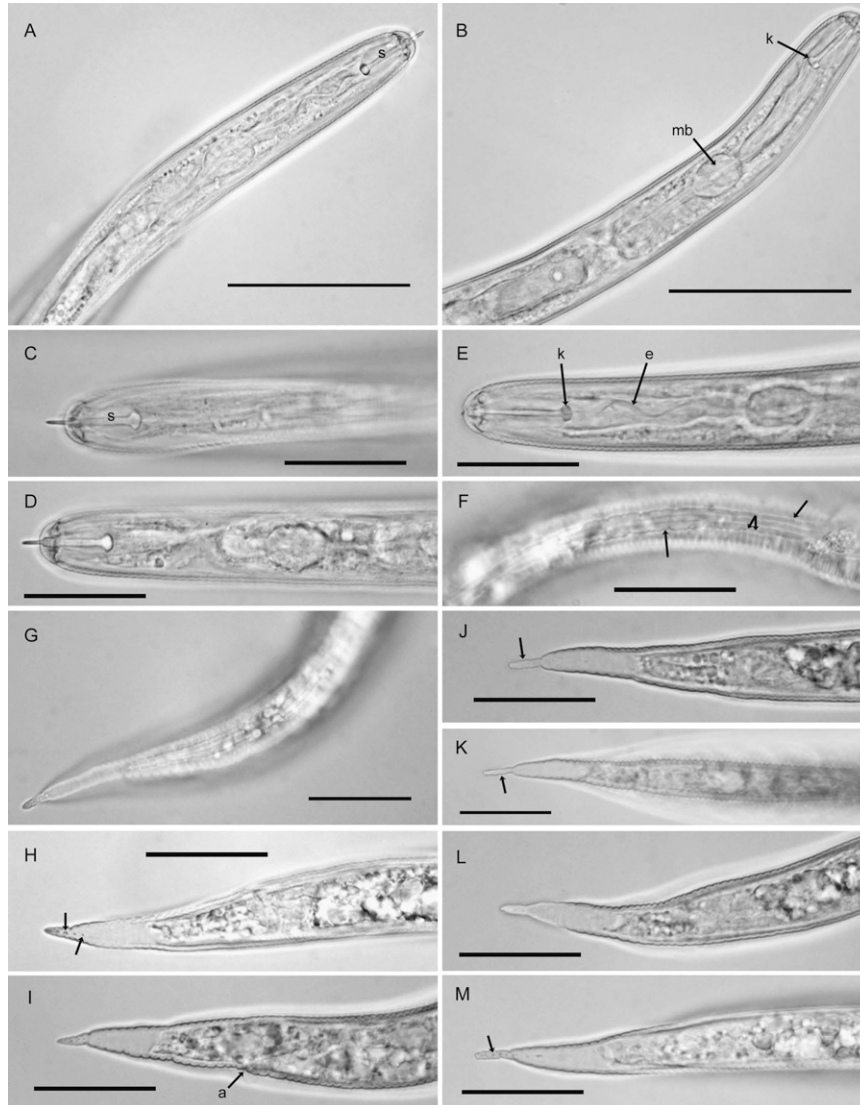


FIG. 6. Photomicrographs of second-stage juveniles of *Globodera ellingtonae* n. sp. from Oregon: A-E, Anterior regions showing stylet and part of esophagus, with k in B, E showing high stylet knobs (arrow), mb = median bulb (arrow) in B, and e = esophagus (arrow) in E. F, showing areolated nature of lateral field with four lines, single arrow = lateral lines, and double arrows = areolation. G-M, tail variations, with H, M (arrows) showing refractive bodies, a = anus (arrow) in I, J-M showing abrupt narrowing of tail with pronounced to slight constriction to peg-like tail terminus. (Scale bars: A, B = 50 μ m; C-M = 25 μ m).

peg-like, finely rounded to pointed terminus (Figs. 5G,I; 6G-M); hyaline tail terminus 24.3 μ m (20-33). Females are characterized by a body length excluding neck of 480 μ m (312-618); stylet length of 21.3 μ m (20-22.5) with 1 head annule and labial disc; heavy punctations on the cuticle; irregular arrangement of bullae-like vulval bodies and short vulval slit 7.8 μ m (7.5-8). Males have a stylet length of 22.9 μ m (21-25); spicule measures 33.5 μ m (30-37) with a pointed thorn-like tip. Cysts are spherical to sub-spherical; light to dark brown with heavy punctations on the cuticle; circumfenestrate; number of cuticular ridges between vulva and anus 13 (10-18) and Granek's ratio 2.4 (1.7-3).

Globodera ellingtonae n. sp. shares similarities with *G. pallida* (Stone, 1973) Behrens, 1975, *G. rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959, *G. mexicana*

Subbotin, Mundo-Ocampo and Baldwin, 2010, and *G. tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975 *sensu* Stone, 1983 (comparative data in Table 3 and differentiation given below for closely related species). It differs from all these species in the shape of the J2 tail, which tapers uniformly but abruptly narrows with a constriction near the posterior third of the hyaline portion and ends with a peg-like, finely rounded to pointed terminus. In other *Globodera* spp. the tail tapers gradually to a bluntly rounded to pointed tip without any constriction or peg-like shape in the posterior third of the hyaline portion. The new species is further distinguished from *G. pallida* in having a shorter stylet mean in J2, females, and males [length 20.9 μ m (19-22.5) *vs.* 23.5 μ m (22.5-25.0) in J2, length 21.3 μ m (20-22.5) *vs.* 27.4 \pm 1.1 in females, length 22.9 μ m (21-25)

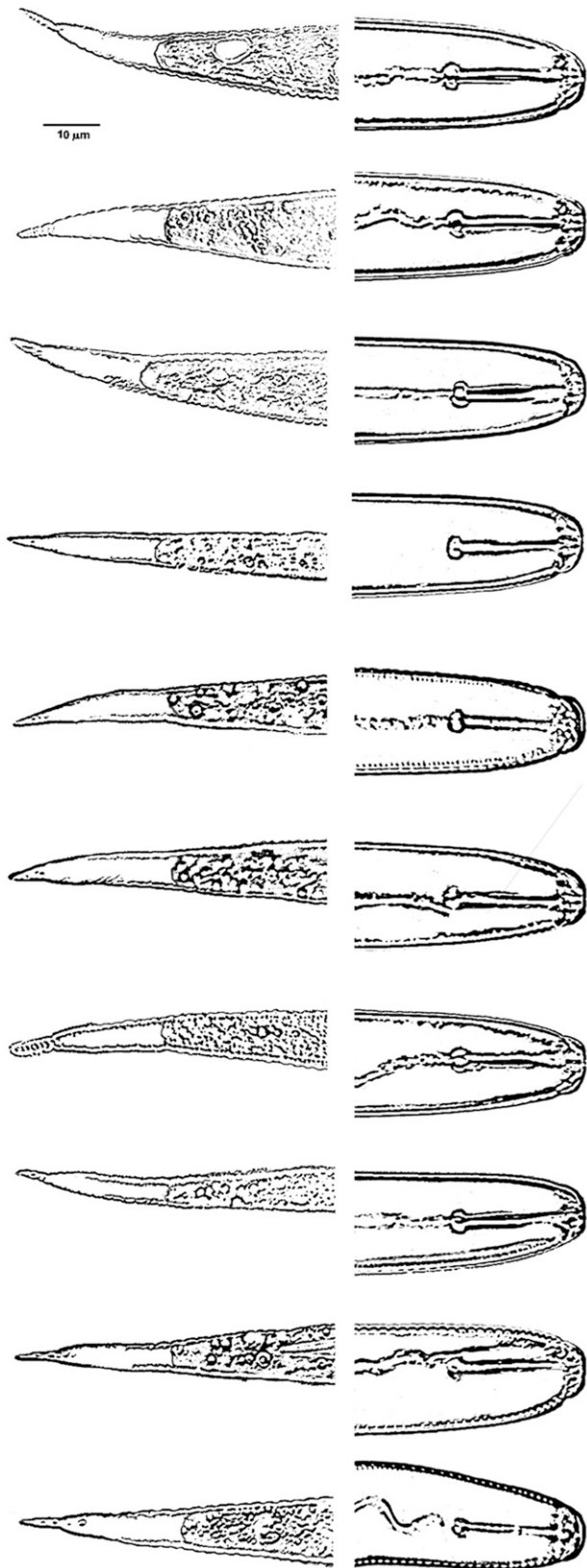


FIG. 7. Heads and tails of second stage juveniles of *Globodera ellingtonae* n. sp. from Oregon.

vs. 27.5 ± 1.0 in males]. The new species differs from *G. rostochiensis* in having J2, females, and males with a shorter stylet mean [length $20.9 \mu\text{m}$ (19-22.5) *vs.* $21.5 \mu\text{m}$ (18.9-23.5), length $21.3 \mu\text{m}$ (20-22.5) *vs.* 23.0 (22-24), length $22.9 \mu\text{m}$ (21-25) *vs.* $25.9 \mu\text{m}$ (25-27), respectively]; cysts have a longer mean fenestra [length $27.4 \mu\text{m}$ (20-42.5) *vs.* $16.3 \mu\text{m}$ (8-20.7)], smaller mean Granek's ratio [2.3 (1.6-3.0) *vs.* 3.9 (2-7)], and fewer number of mean cuticular ridges between the anus and vulva [13 (10-18) *vs.* 18 (16-24)]; the male spicules have a pointed, thorn-like tip *vs.* a bluntly rounded tip, and the J2 hyaline tail terminus has a large number of refractive bodies (4-7) *vs.* (3-4). The new species differs from *G. mexicana* in having J2, females and males with a shorter mean stylet [length $20.9 \mu\text{m}$ (19-22.5) *vs.* $23.3 \mu\text{m}$ (20-27), length $21.3 \mu\text{m}$ (20-22.5) *vs.* $24.7 \mu\text{m}$ (23.2-28.8), and length $22.9 \mu\text{m}$ (21-25) *vs.* $26.7 \mu\text{m}$ (22-29.5), respectively]; cysts have more cuticular ridges between the anus and vulva [13 (10-18) *vs.* 7 (5-9)], and longer mean fenestra [length $27.4 \mu\text{m}$ (20-42.5) *vs.* $20.9 \mu\text{m}$ (15-28.5)]; and spicules in males have a thorn-like tip *vs.* a finely rounded tip.

Globodera ellingtonae n. sp. differs from *G. tabacum tabacum* in possessing cysts with a mean Granek's ratio of more than 2.3 *vs.* 1.6, and a mean of more cuticular ridges between vulva and anus [13 (10-18) *vs.* 8 (5-15)], and with a cyst wall lacking a network-like pattern *vs.* a network-like pattern with parallel anal-fenestral ridges; the J2 have a mean stylet length of less than $21 \mu\text{m}$ *vs.* $23.1 \mu\text{m}$; female stylet length is $21.3 \mu\text{m}$ *vs.* $22.4 \mu\text{m}$; males have shorter mean stylet lengths $22.9 \mu\text{m}$ (21-25), *vs.* $26 \mu\text{m}$ (24-27)]; spicules have a pointed, thorn-like tip *vs.* a finely rounded tip. The new species differs from *G. tabacum solanacearum* in that cysts have more cuticular ridges between vulva and anus [13 (10-18) *vs.* 7 (5-9)], the cyst wall lacks a network-like pattern or ridges are close and irregular around fenestra *vs.* more widely separated ridges and grooves of cyst wall that form a circular network-like pattern around the fenestra. The J2, females and males have a shorter mean stylet length of $20.9 \mu\text{m}$ *vs.* 24.0 , $21.3 \mu\text{m}$ *vs.* 26.2 , and $22.9 \mu\text{m}$ *vs.* 26.3 , respectively; spicules have a pointed, thorn-like tip *vs.* a finely rounded tip; and perineal tubercles in females are clumped *vs.* solitary. The new species differs from *G. tabacum virginiae* by possessing cysts with shorter mean body length excluding neck ($599 \mu\text{m}$ *vs.* $749 \mu\text{m}$), more cuticular ridges between vulva and anus [13 (10-18) *vs.* 7 (6-8)], irregular anal-fenestral ridges *vs.* a maze-like pattern, anus distinct *vs.* small and indistinct, and slightly smaller mean Granek's ratio [2.3 (1.6-3.0) *vs.* 2.7 (1.4-4.2)]; the J2, females and males have a shorter mean stylet length ($20.9 \mu\text{m}$ *vs.* 23.6 , $21.3 \mu\text{m}$ *vs.* 25.0 , and $22.9 \mu\text{m}$ *vs.* 26.6 respectively); perineal tubercles in females are clumped *vs.* solitary; spicules have a pointed, thorn-like tip *vs.* a finely rounded tip.

Etymology: The species is named for Donna Ellington in honor of 40 years of outstanding service to the

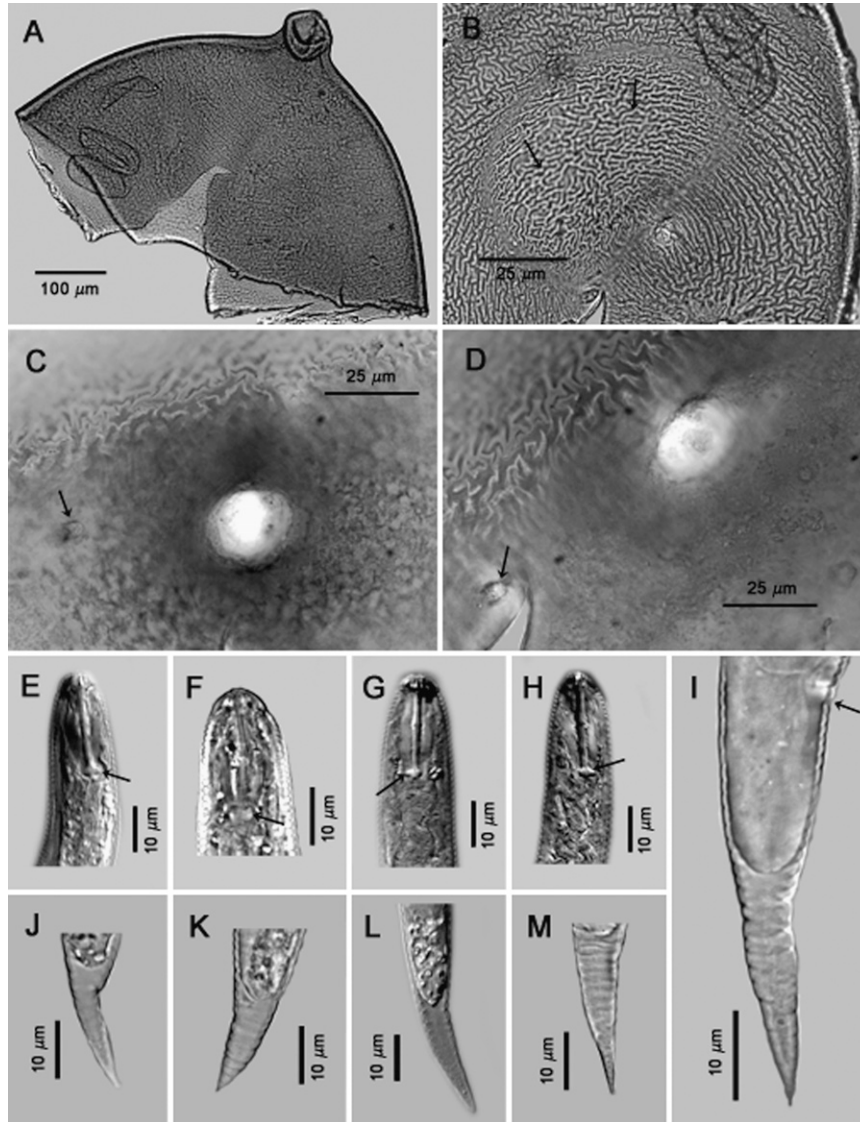


FIG. 8. Photomicrographs of cysts and second-stage juveniles of *Globodera ellingtonae* n. sp. from Idaho: A, anterior region. B-D, anal-vulval regions with B and C-D (arrows) showing cyst wall pattern and anal areas respectively. E-H, anterior and posterior regions of second-stage juveniles with E-H (arrows) showing stylet knobs, with DIC images of E,G,H. I-M, tails with variable termini, DIC image of L. Tail in I shows anal area (arrow). (Modified from Skantar et al., *Phytopathology*, 2011).

taxonomy program of the ARS Nematology Laboratory at Beltsville, Maryland.

DNA sequence analysis: The initial nematode samples obtained from Powell Butte, Oregon showed a high degree of sequence variation in the ITS-rRNA region, initially indicated by the fact that sequencing directly from PCR products gave many ambiguous base calls. Examination of additional cloned amplicons was carried out in order to further explore the extent of ITS rRNA copy variation within this species. A total of 14 clones were obtained from four juveniles of *G. ellingtonae* n. sp. originally isolated from Oregon. Subsequently, 28 clones were generated from five juveniles that exhibited variations in tail morphology, although no correlation between the type of ITS sequence and tail morphology was found (data not shown). Twenty-seven clones were

sequenced from eight juveniles originating from four cysts (two J2 from each) that had been obtained from greenhouse cultures. A total of 11 clones, averaging two per specimen, were sequenced from 5 juveniles of the Idaho isolate #347. As described previously (Skantar et al., 2011), one identical sequence was obtained from direct sequencing of four ITS PCR products from Idaho isolate #167. The sequences ranged in size from 923 to 978 bp, with intraspecific divergence as high as 2.8% (Skantar et al., 2011). The most significant difference among them was a 27 bp deletion detected in three clone sequences amplified from separate juveniles of the original isolate as well as a greenhouse-reproduced cyst.

Phylogenetic analysis: The distinct phylogenetic position of *G. ellingtonae* relative to an extensive array of *G. rostochiensis*, *G. pallida*, and *G. tabacum* populations was

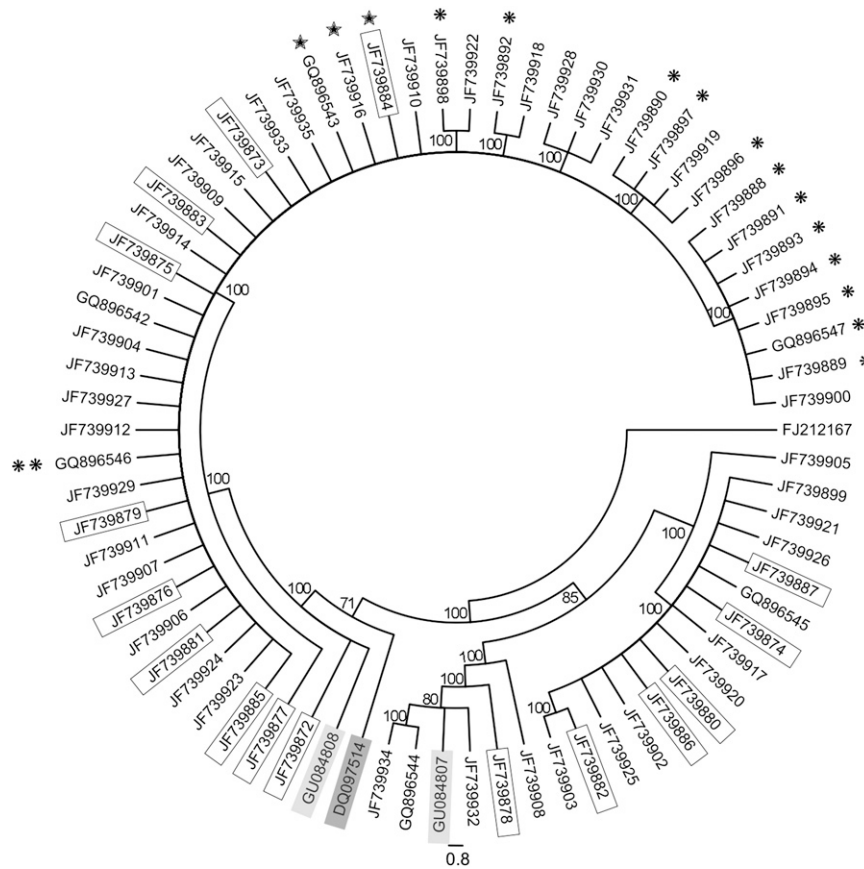


FIG. 10. Maximum parsimony tree of aligned ITS-rRNA gene sequences for species of *Globodera ellingtonae* n. sp with *G. rostochiensis* sequence FJ212167 used as the outgroup. Bootstrap support values from 1000 replicates are indicated on the branches. *Globodera* sp. sequences from Argentina and Chile are shaded in dark and light boxes, respectively. Cloned ITS rRNA gene sequences from the initial Oregon sample are in regular type; sequences obtained from juveniles reproduced in the laboratory are boxed; from Idaho isolate 347 are marked by a single asterisk; a single PCR amplicon sequence from Idaho isolate 167 is marked by two asterisks. Sequences containing a 27 bp deletion are marked with stars.

G. rostochiensis, and *G. pallida* (European and South American subclades 1 – 5). For *G. ellingtonae* n. sp., Bsh 1236I digestion patterns were of two types. Clone sequences with type A patterns were shared with *Globodera* sp. sequences from Chile (GU084808) and *G. rostochiensis*. The Idaho #167 sequence was slightly shorter at either end, due to the lack of reliable base calls close to ends of the amplicons, but the *in silico* patterns otherwise matched Type A. Type B patterns of *G. ellingtonae* n. sp. were the same as for *Globodera* sp. Chile (GU084807) and *G. pallida* subclades 1, 3, and 6. *Globodera tabacum* shared only individual bands with Bsh 1236I patterns A or B. Virtual Rsa I digestion of *G. ellingtonae* n. sp. also gave 2 types of patterns, with types A and B shared with *Globodera* sp. sequences from Chile. The Hinf I digestion products predicted for *G. ellingtonae* n. sp. fell into three possible patterns. Type A was only found in *G. ellingtonae* n. sp. and *Globodera* sp. from Chile; Type B was shared with *G. pallida*, *G. mexicana*, and other *Globodera* sp.; Type C matched *G. tabacum* and *G. rostochiensis*.

PCR-RFLP of the ITS rRNA region is an inexpensive, commonly used diagnostic assay for distinguishing PCN species. Bsh 1236I was previously shown to discriminate

G. pallida, *G. rostochiensis*, and *G. tabacum* (Thiéry and Mugniéry, 1996; Skantar et al., 2007). Previous RFLP analysis of the Powell Butte, OR samples showed visible Bsh 1236I fragment sizes of approximately 820, 500, 342, and 130 bp (Skantar et al., 2011 and *data not shown*), matching some of the sizes predicted for A or B type patterns by the *in silico* analysis (Table 4). Rsa I fragments of 610, 223, 161, 123, and 96 bp were also observed in gels (*not shown*), and closely match the larger sizes predicted from the sequence analysis. Fragments not observed in the gel experiments may have been present at levels too low to be detected. While some of the sequence variation seen in the cloned PCR amplicons of *G. ellingtonae* could reasonably be attributed to errors introduced during PCR, cloning, or sequencing, the patterns representing major and minor sequence types are clearly present in the J2 and can be detected by PCR-RFLP. The presence of ITS haplotypes in *G. pallida* samples from subclade I was also reported by Subbotin et al. (2011). It was proposed that a higher level of rRNA gene heterogeneity may exist for some *Globodera* isolates, or that natural hybrids may underlie unusual diagnostic RFLP profiles. As more populations are examined from South America

TABLE 1. Morphometrics (in μm) of cysts and white females of *Globodera ellingtonae* from Oregon.

Character	Cysts			White females		
	n	Mean \pm SD	Range	n	Mean \pm SD	Range
Body length, excluding neck (L)	20	599 \pm 95	470-815	10	480 \pm 91	312-618
Body width (W)	20	568 \pm 112	415-792	10	409 \pm 140	178-585
L/W ratio	20	1.07 \pm 0.11	0.90-1.22	10	1.25 \pm 0.27	0.96-1.75
Neck length	19	105 \pm 27.4	60-164	10	136 \pm 46.3	85-257
Neck width	19	67.3 \pm 9.22	50-84	10	68.6 \pm 12.9	50-84
Stylet length	-	-	-	8	21.3 \pm 1.07	20.0-22.5
DGO	-	-	-	5	5.1 \pm 0.22	5.0-5.5
Vulval slit length	-	-	-	4	7.88 \pm 0.25	7.5-8.0
Distance from anus to nearest edge of fenestra	23	64.5 \pm 10.3	50-85	4	50.5 \pm 4.20	45-55
Fenestra length	22	27.4 \pm 5.44	20.0-42.5	-	-	-
Cuticular ridges between vulva and anus	23	13.0 \pm 2.31	10-18	4	15.5 \pm 3.32	12-20
Granek's ratio ^a	22	2.37 \pm 0.37	1.69-3.00	-	-	-

^a As modified by Hesling (1973).

and elsewhere, it seems likely that this phenomenon will become more commonplace.

A number of real time (RT) PCR methods have been developed for diagnostics of PCN (Madani et al., 2008; Quader et al., 2008). A multiplex RT-PCR assay with species-specific TaqMan primer-probe sets was recently employed for the simultaneous detection and identification of *G. pallida*, *G. rostochiensis*, and *G. tabacum* (Nakhla et al., 2010). When *G. ellingtonae* n. sp. samples from Oregon were subjected to this test, no fluorescence was detected by any of the primer-probe combinations. This assay, which has become a standard protocol for screening PCN survey samples from throughout the U.S., has thus far resulted in no false positive detections involving *G. ellingtonae* n. sp. The higher degree of specificity conferred by the presence of species-specific TaqMan probes as well as primers may make this test more robust than the conventional PCR-RFLP.

Analysis of the 28S rRNA D2-D3 expansion segment: The sequence for 28S large subunit rRNA D2-D3 expansion regions obtained from *G. ellingtonae* n. sp. was 749 bp in length (JN712217). While relatively few such sequences from *Globodera* species were available for comparison, an alignment of 663 bp was made from 20 sequences, including *G. pallida*, *G. rostochiensis*, *G. tabacum*, and *G. millefolii* (not shown). The greatest similarity of *G. ellingtonae* n. sp. was with *G. tabacum* from Connecticut, U.S. (GQ294492), differing by only 2 bp. Compared with a limited number of available *G. pallida* sequences, Oregon *G. ellingtonae* n. sp. differed by 4bp from a Newfoundland, Canada, population (GQ294489), by 8 bp from the original *G. pallida* population isolated from Idaho (JN712221), and by 14 bp from sequence AY592991, representing a *G. pallida* population from the Netherlands. Compared to *G. rostochiensis*, the Oregon population of *G. ellingtonae* n. sp. differed by 4 bp from identical sequences originating from Quebec and

TABLE 2. Morphometrics (in μm) of males and second-stage juveniles of *Globodera ellingtonae* from Oregon.

Character	Second-stage juveniles			Males		
	n	Mean \pm SD	Range	n	Mean \pm SD	Range
Body length	106	450 \pm 28	365-515	20	981 \pm 97	787-1150
Stylet length	108	20.9 \pm 0.85	19.0-22.5	20	22.9 \pm 1.13	21-25
Height of stylet knobs	-	-	-	15	2.57 \pm 0.18	2.5-3.0
Width of stylet knobs	-	-	-	15	3.83 \pm 0.45	3.5-5.0
DGO	22	7.5 \pm 0.62	6.0-8.5	9	3.44 \pm 0.81	2.5-5.0
Maximum body width	46	20.0 \pm 0.58	18-21	20	37.3 \pm 2.75	32-43
a	46	22.9 \pm 1.38	18.25-25.56	20	26.3 \pm 1.32	23.75-28.72
Distance from anterior end to basal bulb	47	153.8 \pm 8.93	138-180	20	145.2 \pm 12.6	120-160
b	47	2.98 \pm 0.22	2.35-3.42	20	6.81 \pm 0.92	5.25-8.63
tail length	93	46.7 \pm 3.45	39-55	19	3.74 \pm 0.86	2.5-5.0
c	90	9.69 \pm 0.74	8.06-12.25	19	278.3 \pm 77.6	164-432
Hyaline tail terminus length	106	24.3 \pm 2.66	20.0-32.5	-	-	-
Anal body width	38	12.3 \pm 0.82	10.0-13.5	-	-	-
c'	38	3.68 \pm 0.19	3.31-4.00	-	-	-
Refractive bodies on tail terminus	53	5.45 \pm 0.50	5-6	-	-	-
Spicule length	-	-	-	20	33.5 \pm 1.94	30-37
Gubernaculum length	-	-	-	16	11.5 \pm 1.70	8-15
Lateral lines	40	4 \pm 0	4	20	4 \pm 0	4

TABLE 3. Morphological and morphometric characters useful for identification of selected *Globodera* species.^a

	Cysts													
	White females					Males								
	<i>ellingtonae</i> n. sp.	<i>rostochiensis</i>	<i>pallida</i>	<i>tabacum</i> <i>tabacum</i>	<i>tabacum</i> <i>virginiae</i>	<i>tabacum</i> <i>solanacearum</i>	<i>mexicana</i>	<i>ellingtonae</i> n. sp.	<i>rostochiensis</i>	<i>pallida</i>	<i>tabacum</i> <i>tabacum</i>	<i>tabacum</i> <i>virginiae</i>	<i>tabacum</i> <i>solanacearum</i>	<i>mexicana</i>
Body length, excluding neck (L)	599 ± 95 (470-815)	585 (445-990)	578 (420-748)	536 (337-754)	749 (464-937)	699 (447-907)	684	480 ± 91 (312-618)	520 (420-640)	-	464 (327-688)	536 ± 86	488	556
Body width (W)	568 ± 112 (415-792)	505 (250-810)	535 (400-685)	469 (292-812)	532 (383-713)	500 (375-731)	635 (429-800)	409 ± 140 (178-585)	340 (270-430)	-	310 (206-516)	450 ± 68 (313-614)	430 (293-614)	484 (350-670)
L/W ratio	1.07 ± 0.11 (0.90-1.22)	1.19 (0.94-1.8)	-	1.0 ± 0.1 (0.9-1.3)	1.1 ± 0.1 (1.0-1.3)	1.1 ± 0.1 (1.0-1.5)	-	1.25 ± 0.27 (0.96-1.75)	1.5 (1.2-2.0)	-	22.4 (18.5-24.1)	25.0 ± 1.6 (21-29)	26.2 (21-30)	24.7 (23.2-28.8)
Stylet length	-	-	-	-	-	-	-	21.3 ± 1.07 (20.0-22.5)	23.0 (22.0-24.0)	27.4 ± 1.1	5.5 (3.8-8.0)	6.1 ± 1.8 (3-11)	6	6.02 (3.0-10.5)
DGO	-	-	-	-	-	-	-	5.1 ± 0.22 (5.0-5.5)	6.03 (5.7-7.0)	5.4 ± 1.1	-	-	-	-
Distance from anus to nearest edge of fenestra	64.5 ± 10.3 (50-85)	62.5 (29-116)	51.8 (30-80)	44.8 ± 7.9 (29.4-67.2)	51.5 (28-85)	48.0 (30.8-84.0)	58.6 (34-110)	-	-	-	-	-	-	-
Fenestra length	27.4 ± 5.44 (20.0-42.5)	16.3 (8.0-20.7)	23.7 (17.5-45.0)	27.6 ± 3.2 (21.0-36.4)	19.3 (13.0-33.6)	23.0 (16.0-33.6)	20.9 (15.2-28.5)	-	-	-	-	-	-	-
Cuticular ridges between vulva and anus	13.0 ± 2.31 (10-18)	18.2 (16-24)	12 (7-17)	8.8 ± 2.1 (5-15)	8.3 ± 1.0 (6-8)	7.2 (5-9)	-	-	-	-	-	-	-	-
Granek's ratio	2.37 ± 0.37 (1.69-3.00)	3.94 (2.0-7.0)	2.24 (1.2-3.6)	1.60 ± 0.3 (1.0-2.4)	2.70 (1.4-4.2)	2.5 ± 0.4 (1.4-2.9)	2.8 (1.66-5.33)	-	-	-	-	-	-	-

	Second-stage juveniles													
	White females					Males								
	<i>ellingtonae</i> n. sp.	<i>rostochiensis</i>	<i>pallida</i>	<i>tabacum</i> <i>tabacum</i>	<i>tabacum</i> <i>virginiae</i>	<i>tabacum</i> <i>solanacearum</i>	<i>mexicana</i>	<i>ellingtonae</i> n. sp.	<i>rostochiensis</i>	<i>pallida</i>	<i>tabacum</i> <i>tabacum</i>	<i>tabacum</i> <i>virginiae</i>	<i>tabacum</i> <i>solanacearum</i>	<i>mexicana</i>
Body length	450 ± 28 (365-515)	440 (366-502)	468 (380-533)	489 (410-580)	511 (419-661)	503 (390-459)	469 (333-587)	981 ± 97 (787-1150)	1139 (890-1270)	1198 ± 104	1123 (710-1355)	1164 ± 98 (850-1325)	1108 (750-1450)	1150 (800-1300)
Stylet length	20.9 ± 0.86 (19.0-22.5)	21.5 (18.9-23.5)	23.5 (22.5-25.0)	23.1 (19.8-26.0)	23.6 (19-28)	24.0 (22.0-26.6)	23.3 (20-27)	22.9 ± 1.13 (21-25)	25.9 (25-27)	27.5 ± 1.0	26.0 (24-27)	26.6 ± 0.9 (24-29)	26.3 (24-27)	26.7 (22.0-29.5)
Stylet knobs	Rounded to anteriorly projected	Rounded	Anteriorly projected	Rounded to anteriorly projected	Rounded to anteriorly projected	Rounded to anteriorly projected	Anteriorly projected	Rounded to anteriorly projected	Rounded	Posteriorly sloped	Rounded to laterally directed	Rounded to posteriorly sloped	Rounded to posteriorly sloped	Posteriorly sloped
DGO	-	-	-	-	-	-	-	3.44 ± 0.81 (2.5-5.0)	5.85 (5.3-7.0)	3.4 ± 1.0	3.6 (2.0-5.5)	3.5 ± 0.8 (2.0-6.0)	2.3 (1.5-4.0)	3.1 (1.4-4.8)
Tail length	46.7 ± 3.45 (39-55)	47.1 (36.4-57.0)	51.0 (40-57)	51.3 (44.1-60.3)	51.2 (34.0-63.7)	50.5 (33.0-61.8)	54 (44.2-74.2)	-	-	-	-	-	-	-
Hyaline tail terminus length	24.3 ± 2.66 (20.0-32.5)	24.3 (18.0-30.0)	26.2 (20-31)	27.2 (17.1-31.0)	27.2 (19-35)	24.4 (16-30)	24.5 (19.8-28.8)	-	-	-	-	-	-	-
Spicule length	-	-	-	-	-	-	-	33.5 ± 1.94 (30-37)	35.3 (32-39)	36.3 ± 4.1	26.34 (26-34)	32.8 ± 1.3 (30-35)	32.9 (27.8-36.3)	33.9 (27.8-36.3)
Gubernaculum length	-	-	-	-	-	-	-	11.5 ± 1.70 (8-15)	11.2 (10-14)	11.3 ± 1.6 (9-12)	-	-	-	9.2 (8.2-11.0)

^a Data are in μm and are given as means or mean \pm SD (outside parentheses) and ranges of individual measurements (inside parentheses). *Globodera ellingtonae* from Oregon: this paper; all others: Subbotin *et al.* (2010a). *G. rostochiensis*, *pallida*, *tabacum*: composites of populations listed in Subbotin *et al.* (2010a); reported as mean of means and range of individual measurements if given (or, if only one population was measured for a certain character, mean \pm SD). *G. rostochiensis* females: body length (L) includes neck.

TABLE 4. Approximate sizes (in bp) of restriction fragments estimated from *in silico* digestion of *Globodera* spp. ITS rDNA sequences, including the amplified region between primers TW81 and AB28.

Species	Accession #	Uncut size	Bsh 1236I	Hinf I	Rsa I
<i>Globodera allingtonae</i> Oregon	GQ896542	976	826, 130, 20 (A)	605, 152, 145, 74 (A)	584, 162, 124, 97, 9 (A)
Oregon	GQ896543	950	800, 130, 20 (A)	579, 152, 145, 74 (A)	558, 162, 124, 97, 9 (A)
Oregon	GQ896544	977	486, 342, 129, 20 (B)	751, 152, 74 (B)	584, 222, 162, 9 (B)
Oregon	GQ896545	978	486, 342, 130, 20 (B)	904, 74 (C)	595, 221, 162 (B)
Idaho #167	GQ896546	923	787*, 116*, 20 (A)	591*, 152, 145, 35* (A)	570*, 124, 123*, 97, 9 (A)
Idaho #347	GQ896547	976	826, 130, 20 (A)	758, 152, 74 (B)	584, 162, 124, 97, 9 (A)
<i>Globodera</i> sp., Antofagasta, Chile	GU084808	984	828, 127, 20 (A)	604, 152, 145, 74 (A)	583, 162, 124, 97, 9 (A)
<i>Globodera</i> sp., Antofagasta, Chile	GU084807	984	485, 342, 127, 20 (B)	748, 152, 74 (B)	582, 221, 162, 9 (B)
<i>Globodera</i> sp., Argentina	DQ097514	930	486, 294*, 130*, 20 (B)	752*, 151, 27* (B)	586*, 221, 114*, 9 (B)
<i>G. pallida</i> North America, Europe, South America		979	484, 342, 130, 20 (B) or 352, 342, 135, 130, 20	753, 152, 74 (B)	586, 384, 9
(subclade 1)**					
Amantani (subclade 3)	EU006705	979	352, 342, 135, 130, 20	753, 152, 74 (B)	586, 384, 9
Taquile (subclade 3)	GU084797	975	486, 342, 127, 20 (B)	749, 152, 74 (B)	316, 266, 222, 162, 9
Huancabamba (subclade 5)	EU006704	969	829, 120, 36, 20	743, 152, 74 (B)	576, 222, 162, 9 (B)
Cusco (subclade 6)	GU084803	976	487, 342, 127, 20 (B)	750, 152, 74 (B)	583, 222, 162, 9 (B)
<i>G. mexicana</i>	EU006707	982	489, 341, 132, 20 (B)	757, 151, 74 (B)	589, 383, 10
<i>G. rostochiensis</i> **		976	826, 130, 20 (A)	902, 74 (C)	584, 221, 162, 9 (B)
<i>G. tabacum</i> **		980	431, 342, 130, 57, 20	906, 74 (C)	587, 222, 162, 9 (B)

* Available sequence is slightly short at either end, altering some fragment sizes.

** Information adapted from Table 2 in Subbotin et al., 2011.

Newfoundland, Canada, (GQ294487, GQ29488); and 9 bp from sequences from Netherlands populations (AY592987, A7592988). There were 12 bp separating *G. ellingtonae* n. sp. from *G. millefolii* (DQ328700). Madani et al. (2010) previously concluded that for *Globodera* spp., low diversity within the D2-D3 expansion segment of 28S rRNA did not allow robust phylogenetic reconstruction within this genus. The >99% similarity of *G. ellingtonae* n. sp. to *G. tabacum* sequences is consistent with this conclusion, and furthers the presumption that this marker also lacks sufficient variability to be useful as a molecular diagnostic for distinguishing *G. pallida*, *G. rostochiensis*, and *G. tabacum*.

The possible existence of an additional species of PCN, particularly one originating from South America, has been the subject of study and speculation for some time (Phillips and Trudgill, 1998; Blok et al., 1998; Subbotin et al., 2000; Rumpfenhorst and Ayub, 2001; Picard et al., 2007; Plantard et al., 2008; Pylypenko et al., 2008). Based on phylogenetic analysis of ITS rRNA and mitochondrial cytochrome *b* (*cytb*) sequences, Subbotin and colleagues (Madani et al., 2010; Subbotin et al., 2011) have proposed possible candidates from among South American populations of *G. pallida* or undescribed species, appearing in ITS rRNA trees within subclades 3, 5, and 6 (see also Table 4, this study). They concluded that the pale potato cyst nematode, *G. pallida*, may represent a species complex, and agreed with Grenier et al. (2001) who emphasized the need to augment molecular data with morphological and biological studies. Based upon molecular data presented here and elsewhere (Skantar et al., 2011; Subbotin et al., 2011), *G. ellingtonae* n. sp. is molecularly more similar to *G. tabacum* and *G. rostochiensis* than to *G. pallida*.

Material representing the two Idaho isolates was in relatively poor condition and all available specimens were used for identification, so no additional material was available for culturing. Since 2006, the USDA Animal and Plant Health Inspection Service and the States have collected and analyzed over 300,000 official samples for *Globodera* from potato fields in Idaho and over 100,000 samples from potato fields in 30 other states; *G. ellingtonae* was not detected in any of these samples (Jonathan Jones, personal communication). Therefore, we lacked the appropriate number of specimens needed for optimal replication of the morphometrics as we had for the Oregon population. This situation warrants some degree of caution with respect to the identity of these Idaho isolates as *G. ellingtonae* n. sp. Nonetheless, the existence of unequivocal molecular similarity between the Oregon and Idaho isolates provides strong support that the three belong to the same species, an inference further supported by the limited morphological and morphometric data from the Idaho isolates. The morphological and molecular description provided here for *G. ellingtonae* n. sp. lies in contrast to the still unresolved morphological status of many South

American populations of *Globodera*, most of which have been described only molecularly. Anthoine et al. (2008) and Grenier et al. (2010) recently highlighted potential problems with the reliability of molecular diagnostics in light of variable South American populations, pointing out that the identities of variable Chilean populations have not yet been validated. Morphological descriptions of these and other South American populations would certainly strengthen the taxonomic foundation needed for traditional diagnosis, which, despite the increased use of molecular approaches, is still an essential part of PCN control programs. Notably, the sequences from the Antofagasta, Chile isolate closely match those from *G. ellingtonae*; however, absent any morphological details, identifying them as such may be premature. Preliminary greenhouse experiments have demonstrated that *G. ellingtonae* n. sp. can reproduce on potato (*Solanum tuberosum*), with further host range and details about virulence anticipated in a separate publication (Zasada and Ingham, *personal communication*). Therefore, this present description provides fundamental information that is needed for morphological and molecular diagnosis of this species to provide continued protection for the potato industry in the U.S. and throughout the world.

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