Morphological and molecular characterisation of the Mexican cyst nematode, *Globodera mexicana* Subbotin, Mundo-Ocampo & Baldwin, 2010 (Tylenchida: Heteroderidae)

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Summary. The Mexican cyst nematode, *Globodera mexicana*, was reported from wild solanaceous plants collected from several locations in Mexico. The analysis of *COI*, *cytb*, ITS rRNA and *hsp90* gene sequences suggested that *G. pallida* and *G. mexicana* are sister species. In this study, 15 new *COI*, three new *cytb*, six new *hsp90*, and two new ITS rRNA gene sequences were provided. Maximal intraspecific *COI* gene sequence diversity for *G. mexicana* was estimated as 9.9%. Morphological description of *G. mexicana* from type and another location was also provided. Considering our current knowledge on findings of *G. mexicana*, it has been suggested that *G. mexicana* originated and diversified in Mexico and the cysts of its ancestor have dispersed to Mexico by passing through the northern Andes and Central America. Future studies of the genus *Globodera* should focus on increased sampling in these regions of Central and South America from wild solanaceous plants to reconstruct a more complete picture of phylogeography and evolution of this genus.

Key words: biogeography, *COI*, *cytb*, *Globodera*, Mexico, phylogeny.

In 1963, during nematological surveys of wild potatoes and solanaceous plants in the Central Highlands of Mexico's Toluca Valley in the state of Mexico and Huamantla Valley in the state of Tlaxcala, many cysts closely resembling potato cyst nematodes were found from *Solanum rostratum* Dunal. Detailed biological and morphological studies made by Campos-Vela (1967) revealed this nematode represented an undescribed species. The data from this work, together with a description of a species named as *Heterodera mexicana*, was presented in Campos-Vela's Ph.D. thesis. Several years later, however, Golden and Ellington (1972) considered *H. mexicana* as a *nomen nudum* as the description of this nematode was presented in a Ph.D. thesis, which could not be considered as a valid publication for the purposes of species nomenclature. Considering that, the Mexican cyst nematode is biochemically and molecularly different from other *Globodera*, Subbotin *et al.* (2010) proposed to re-establish this species by accepting it as a valid species and naming it *G. mexicana*.

Experiments showed that *G. mexicana* was unable to develop on potato (*Solanum tuberosum*), whereas tomato (*Solanum lycopersicum*) was a common host for *G. pallida* and *G. mexicana* (Thiéry *et al.*, 1997). These two species were able to mate and form hybrids (Mugniéry *et al.*, 1992). Results of two-dimensional gel electrophoresis of total proteins (Bossis & Mugniéry, 1993), PCR-ITS-RFLP (Thiéry *et al.*, 1997; Grenier *et al.*, 2002), PCR-RAPD (Thiéry *et al.*, 1997), and satellite DNA sequences (Grenier *et al.*, 2002) showed that the *G. mexicana* was clearly different from all other *Globodera* spp. and shared a high degree of genome similarity with *G. pallida*. Several genes responsible for parasitism and interaction with plants were characterised in *G. mexicana* and compared with those from *G. pallida* (Grenier *et al.*, 2002; Blanchard *et al.*, 2005; Sacco *et al.*, 2009; Stare *et al.*, 2011) and sequences of *cytb* (Picard *et al.*, 2007), ITS rRNA (Picard *et al.*, 2008; Subbotin *et al.*, 2011), and 18S rRNA (Helder *et al.*, 2014) genes were also published. Eight populations of

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G. mexicana, including the topotype population, were recently analysed using ITS rRNA, *cytb* and *COI* gene sequences (Subbotin *et al.*, 2020).

In this study, we provide morphological descriptions of the topotype population of *G. mexicana* and analyse additional populations of this species. Updated phylogenetic networks for *COI* and *cytb* genes and a phylogenetic tree showing relationships within *G. pallida* and *G. mexicana* based on the analysis of *hsp90* gene sequences are also provided.

MATERIAL AND METHODS

Nematode samples. In September 2021, a nematological survey was conducted across Central Mexico. More than 50 soil samples from solanaceous and other plants were collected from several locations. Adult and second-stage juvenile (J2) cyst nematodes were also collected from the type locality of *G. mexicana* in Tlaxcala State, Huamantla, Francisco Villa Tecoac. Cysts and J2 were extracted from soil samples using standard centrifugal-flotation and the Fenwick methods (Fenwick, 1940). Several dozen cysts were obtained from Stone's nematode collection presently kept at Fera Science Ltd., York, UK. They were also included in this study. These cysts were collected during a visit to Mexico in November 1975 (Table 1). For molecular analysis, several other *G. mexicana* populations characterised in a previous publication (Subbotin *et al.*, 2020) were also used.

Morphological study. Males, females and J2 were killed using 8% formalin. The nematodes were then processed to glycerin using a modification of the Seinhorst (1959) method. Morphometrics of certain characters were calculated following schematic drawings made using a drawing tube mounted on an American Optical Compound Microscope (AO, USA). Light micrographs of cysts and J2 were taken with automatic Lumenera Infinity 2 camera (Japan) attached to a compound Olympus BX51 microscope (Japan) equipped with Nomarski differential interference contrast.

For scanning electron microscopy, nematodes were treated in phosphate buffer for 15 min and dehydrated in an alcohol series (10-100%) for 15 min at each concentration (Cid del Prado Vera *et al.*, 2012). The specimens were critical point-dried and coated with gold-palladium before observation under a scanning electron microscope, Jeol JSM-6390 (Japan) at 10 kV.

DNA Extraction, PCR and sequencing. Cysts were soaked for 10-20 min in double distilled water (DDW). One cyst was placed in 20 µl DDW on a glass slide, punctured by a needle under a dissecting microscope to release J2 and eggs, which were then cut using a stainless-steel dental needle under a stereomicroscope. DNA was extracted using a standard protocol with proteinase K (Subbotin *et al.*, 2020). Fragments of nematodes in the water suspension were transferred into a 0.2 ml Eppendorf tube and 3 µl proteinase K (600 µg ml^{-1}) (Promega) and 2 µl 10X PCR buffer (*Taq* PCR Core Kit, Qiagen) were added to each tube. The tubes were incubated at 65°C (1 h) and 95°C (15 min) consecutively. After incubation, the tubes were centrifuged and kept at –20°C until use.

PCR and sequencing were performed as described by Subbotin *et al.* (2020). Several primer sets were used: the forward Het-coxiF (5'-TAG TTG ATC GTA ATT TTA ATG G-3') and the reverse Het-coxiR (5'-CCT AAA ACA TAA TGA AAA TGW GC-3') primers for amplification of the partial *COI* gene; the forward Het-cytbF2 (5'-CAR TAT TTR ATR TTT GAR GT-3') and reverse Het-cytbR3 (5'-ACH ARR AAR TTR ATY TCC TC-3') primers for amplification of the partial *cytb* gene; the forward TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and the reverse AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') for amplification of the ITS1-5.8S-ITS2 rRNA gene; and, the forward U831 (5'-AAY AAR ACM AAG CCN TYT GGAC-3') and the reverse L1110 (5'-TCR CAR TTV TCC ATG ATR AAV AC-3') for amplification of the *hsp90* gene. The PCR products of several samples were cloned into the pGEM-T vector and transformed into JM109 High Efficiency Competent Cells (Promega, USA). Sequencing was performed by Genewiz (San Francisco (CA), USA). New sequences were submitted in GenBank under accession numbers indicated in Table 1.

Phylogenetic analysis. Alignments of the *COI*, *cytb*, ITS rRNA and *hsp90* gene sequences were created using ClustalX 1.83 (Chenna *et al.*, 2003) with default parameters. New sequences were aligned with corresponding published gene sequences (Subbotin *et al.*, 2020). Several alignments were created: *i*) *COI* gene alignment containing sequences of *G. mexicana*; *ii*) *cytb* gene alignment containing sequences of *G. mexicana*; *iii*) ITS rRNA gene alignment containing sequences of *G. mexicana* and some sequences of other *Globodera* species parasitising solanaceous plants;

Table 1. Populations of *Globodera mexicana* used in this study.

Location	GPS coordinates	Plant-host	Sample code	Haplotypes	GenBank accession number				Source
					ITS rRNA	*COI*	*cytb*	*hsp90*	
Mexico, State of Mexico, Juchitepec County	19.11159 -98.93599	*Solanum pubigerum*	CD3563, M32	GmexCOI3/ GmexCb4	OQ318153	OQ318069-OQ318071	OQ320750	OQ332473	I. Cid del Prado Vera
Mexico, State of Mexico, Santiago Cuaula	19.57835 -98.64185	*S. nigrescens*	CD3564, M49	GmexCOI5/ GmexCb5	OQ318154	OQ318074-OQ318076, OQ318079	OQ320748	OQ332475	I. Cid del Prado Vera
Mexico, State of Tlaxcala, Santiago Cuaula	19.57835 -98.64185	*S. pubigerum*	CD3565, M50	GmexCOI5	–	OQ318068, OQ318072, OQ318073, OQ318077, OQ318078	–	–	I. Cid del Prado Vera
Mexico	Unknown	Unknown	CD3602	GmexCOI8/ GmexCb6	–	OQ318080-OQ318082	OQ320749	–	V. Orlando
Mexico, State of Mexico, Texcoco, El jardín de Tequexquinahuc	19.45532 -98.77861	*S. nigrum*	CD2821	GmexCOI2	MN258870	MN095874, MN095875	–	OQ332472, OQ332474	Subbotin *et al.* (2020)
Mexico, State of Mexico, Amecameca, San Diego, Huehuecalco	19.09263 -98.74487	*S. stoloniferum*	CD2809	GmexCOI5	–	MN095867	–	OQ332471	Subbotin *et al.* (2020)
Mexico, State of Mexico, San Miguel de la Victoria	20.08520 -99.62422	*S. nigrum*	CD2814	GmexCOI1	MN258868	MN095877	–	OQ332476	Subbotin *et al.* (2020)
Mexico, Tlaxcala State, Huamantla, Francisco Villa Tecoac	19.38455 -97.92855	*S. rostratum*	CD2862	GmexCOI5, GmexCOI7, GmexCOI6	MN258871	MN095865, MN095866, MN095872	–	–	Subbotin *et al.* (2020)

Fig. 1. Cyst and females of *Globodera mexicana* from the type locality. Scale bar = 500 µm.

and *iv*) *hsp90* gene alignment containing sequences of *G. mexicana* and some *G. pallida* sequences. Alignments of ITS rRNA and *hsp90* gene sequences were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) as described by Subbotin *et al.* (2020). The best fit models of DNA evolution were obtained using the program jModelTest 0.1.1 (Posada, 2008) with the Akaike Information Criterion. The alignments for *COI* and *cytb* gene sequences were used to construct phylogenetic network estimation using statistical parsimony (SP) as implemented in POPART software (http://popart.otago.ac.nz) (Bandelt *et al.*, 1999). The haplotypes were identified based on the SP results. Haplotype numbers are given according to Subbotin *et al.* (2020).

RESULTS

After screening 50 soil samples from solanaceous and other plants in Mexico, cysts and females of *G. mexicana* were found from three samples: CD3563, CD3564 and CD3565 (Table 1).

Morphological characterisation of *Globodera mexicana*. Two populations of this species: one from the type locality and another one from the State of Mexico, Amecameca, San Diego, Huehuecalco were used for morphological description. Table 2 provides measurements of *G. mexicana* of these two populations, populations given by Campos-Vela (1967) and Franco *et al.* (2000) and *G. pallida* for comparison. Some morphological and morphometric characters used for diagnostics of *Globodera* parasitising solanaceous plants are given in Table 3.

Females. Mature white female pear to spherical shape with short neck, less than 120 µm long; in some females a transparent-yellow secretion located around the neck was observed. Lip region with two cephalic rings, the first annuli 4 µm in width and the second 5 µm; cuticle with fine annulation and circumfenestra area lace-like in pattern. Vulval slit 10 µm long and circumfenestra 14 to 15 µm diam. Distance of the border of circumfenestra to anus 18 to 60 µm. Stylet with conus curved dorsally. Procorpus with distortion by the gland's secretion. Metacorpus spherical in shape, in general 25 µm diam. Pharyngeal glands forming a lobe, overlapping intestine; excretory pore posterior to metacorpus.

Table 2. Morphometrics of cysts, second-stage juveniles and males of *Globodera mexicana* and *G. pallida*. All measurements are in μm and in the form: range (mean ± s.d.).

Character \ Population	Mexico, Tlaxcala State, Huamantla, Francisco Villa Tecoac, type locality, *Solanum rostratum*, This study	Mexico, State of Mexico, Amecameca, San Diego Huehuecalco, *Solanum staloniferum*, This study	Mexico, Tlaxcala State, Huamantla, Francisco Villa Tecoac, type locality, Campos-Vela (1967)	Mexico, Mexico City, *Jaltomata procumbens*, (= *G. bravoae*), Franco *et al.* (2000)	*G. pallida*, Handoo *et al.* (2012)
Female					
n	15	12	33	50	–
L	350-645 (514.8±79.1)	400-700 (561.3±93.7)	540-980 (695.5)	152-836 (534±26.7)	–
W	200-472.5 (352.2±78.3)	217.5-542.5 (394.7±98.5)	350.4-670.0 (483.6)	368-620 (487.5±17.7)	–
L/W	1.1-2.0 (1.5±0.3)	1.2-1.8 (1.5±0.2)	–	0.4-1.7 (1.4±0.1)	–
Stylet	22-25 (24.2±1.3)	23-26 (24.3±1.5)	23.2-28.8 (24.7)	21.6-28.0 (24.5±0.7)	27.4±1.1
Length of metacorpus	25-35 (29.8±3.5)	22-35 (29.9±3.9)	–	24.8-52.4 (34.6±1.9)	–
Width of metacorpus	25-30 (28.5±1.9)	25-35 (29.1±3.3)	–	–	–
Cyst					
n	4	6	100	40	–
L	412-520 (450.5±47.7)	312-600 (383.7±107)	671-999 (815)	459-936 (739±35.6)	420-748 (578)
W	322-416 (372±43.6)	310-480 (355±63.5)	429-800 (635)	296-749 (579±40.1)	400-685 (535)
L/W	1.1-1.3 (1.2±0.1)	1.0-1.3 (1.1±0.1)	–	1.1-1.6 (1.3±0.1)	–
Fenestra diam	11.0-27 (20.6±5.9)	15-33 (22.7±4.8)	15.2-28.5 (20.9)	22-35 (27.8±1.1)	17.5-45.0 (23.7)
Anus to nearest fenestral margin distance	35-60 (45.8±9.6)	30-70 (46.5±11.4)	34.2-110.0 (58.6)	34-80 (47.5±4.6)	30-80 (51.8)
Number of ridges between vulva and anus	9-15 (12±2.8)	7-14 (10.4±2.2)	–	8-15 (10.7)	7-17 (12)
Granek ratio	1.8-3.2 (2.3±0.6)	0.8-2.9 (2.1±0.5)	1.7-5.3 (2.8)	1.3-2.7 (1.8)	1.2-3.6 (2.2)
Second-stage juvenile					
n	5	5	125	59	–
L	318-411 (364±41.5)	420-550 (470±0.05)	333-587 (468.5)	442-553 (484.6±6.9)	380-533 (468)
a	18.7-24.2 (22.8±2.3)	20.8-30.5 (25.4±3.5)	18.0-26.0 (23.6)	20.4-31.1 (25.5±0.6)	–
b	4.7-6.8 (5.46±0.9)	5.4-6.2 (5.7±0.4)	2.1-3.7 (2.7)	4.3-4.8 (4.4±0.15)	–
b'	3.4-5.1 (4.2±0.7)	2.8-3.3 (3.0±0.3)	–	2.3-3.1 (2.6±0.1)	–
c	2.6-4.7 (3.9±0.8)	7.4-9.2 (8.2±0.9)	5.7-11.6 (8.6)	7.5-10.2 (8.6±0.1)	–
c’	2.6-4.7 (3.9±0.8)	4.2-5.5 (5.0±0.5)	–	4.2-6.2 (4.9±0.1)	–
Stylet	21-23 (22±0.8)	23-24 (23.6±0.6)	20.0-27.0 (23.3)	18.4-26.8 (22.6±0.5)	22.5-25.0 (23.5)
Stylet knob width	3.0-4.0 (3.8±0.5)	4.0	–	3.6-6.0 (4.3±0.1)	–
Lip region width	8-10 (9.2±0.8)	9.0-10 (9.6±0.6)	–	8.0-11.6 (9.2)	–
Lip region height	4-5 (4.2±0.5)	3.0-5.0 (4.2±0.8)	–	3.2-5.6 (4.3±0.1)	–
DGO	3.0	4.0-7.0 (5.3±1.5)	–	5.6-8.0 (6.8±0.2)	–
Length of metacorpus	8-12 (9.5±1.7)	12-14 (13.3±1.2)	–	–	–
Primordium to anterior end	174-193 (183.5±13.4)	245-250 (247.5±3.5)	140-200 (175.7)	236-305 (270±5.6)	–
W (maximum)	–	–	–	16-24 (19.2±0.6)	–
Excretory pore	–	95-105 (98.3±5.8)	88.0-110.0 (100.4)	–	–
Tail	34-52 (46.2±7.6)	50-63 (56.8±4.9)	44.2-74.2 (54.0)	45-66 (56.2±1.1)	40-57 (51.0)
Hyaline part of tail length	25-31 (28.6±2.5)	25-34 (30.2±4.0)	19.8-28.8 (24.5)	24.8-41.2 (32.2±1.0)	20-31 (26.2)
Male					
n	1	8	33	40	–
L	960	1020-1190 (1100±50)	800-1300 (1150)	819-1186 (1017±26.6)	1198±104
a	26.7	31-39 (36.7±2.5)	28.5-45.1 (35.9)	22.9-41.3 (32.5±1.9)	–
b	–	8.1-9.5 (8.9±0.5)	4.5-7.9 (6.1)	–	–
b'	–	5.7-7.0 (6.4±0.6)	–	–	–
c	192	159.3-278 (233.6±41)	130-547 (216)	134.9-459.7 (268.6±6.2)	–
c’	0.3	0.2-0.6 (0.3±0.1)	–	0.2-0.5 (0.3± 0.01)	–
Stylet	–	24-27 (25.5±0.9)	22-29.5 (26.7)	18.8-28.0 (24.2 ±0.7)	27.5±1.0
Lip region width	10.0	10-12 (10.6±0.7)	–	8.4-12.4 (11.3±0.3)	–
Lip region height	6.0	5.0-10.0 (6.3±1.8)	–	4.4-8.4 (6.2±0.4)	–
DGO	–	3.0-5.0 (4.0±0.8)	–	4.0-6.4 (4.7±0.2)	3.4±1.0
Length of metacorpus	–	12-18 (14.6±1.9)	–	–	–
Excretory pore	120	135-160 (145.4±8.8)	–	96.4-169.9 (141.4±5.0)	–
W (maximum)	36	24-32 (26.8±3.0)	–	24.4-44.0 (32.1±1.6)	–
Testis	–	45-63 (58.6±6.5)	51.2-68.3 (66.4)	22.5-66.4 (44.9±11.9)	–
Tail	5.0	2.0-6.0 (4.3±1.9)	2.0-8.8 (5.8)	2.4-7.6 (4.1±0.4)	–
Spicule length	28	22-34 (30±4.1)	27.8-36.3 (33.9)	24-42 (31.1±1.2)	36.3±4.1

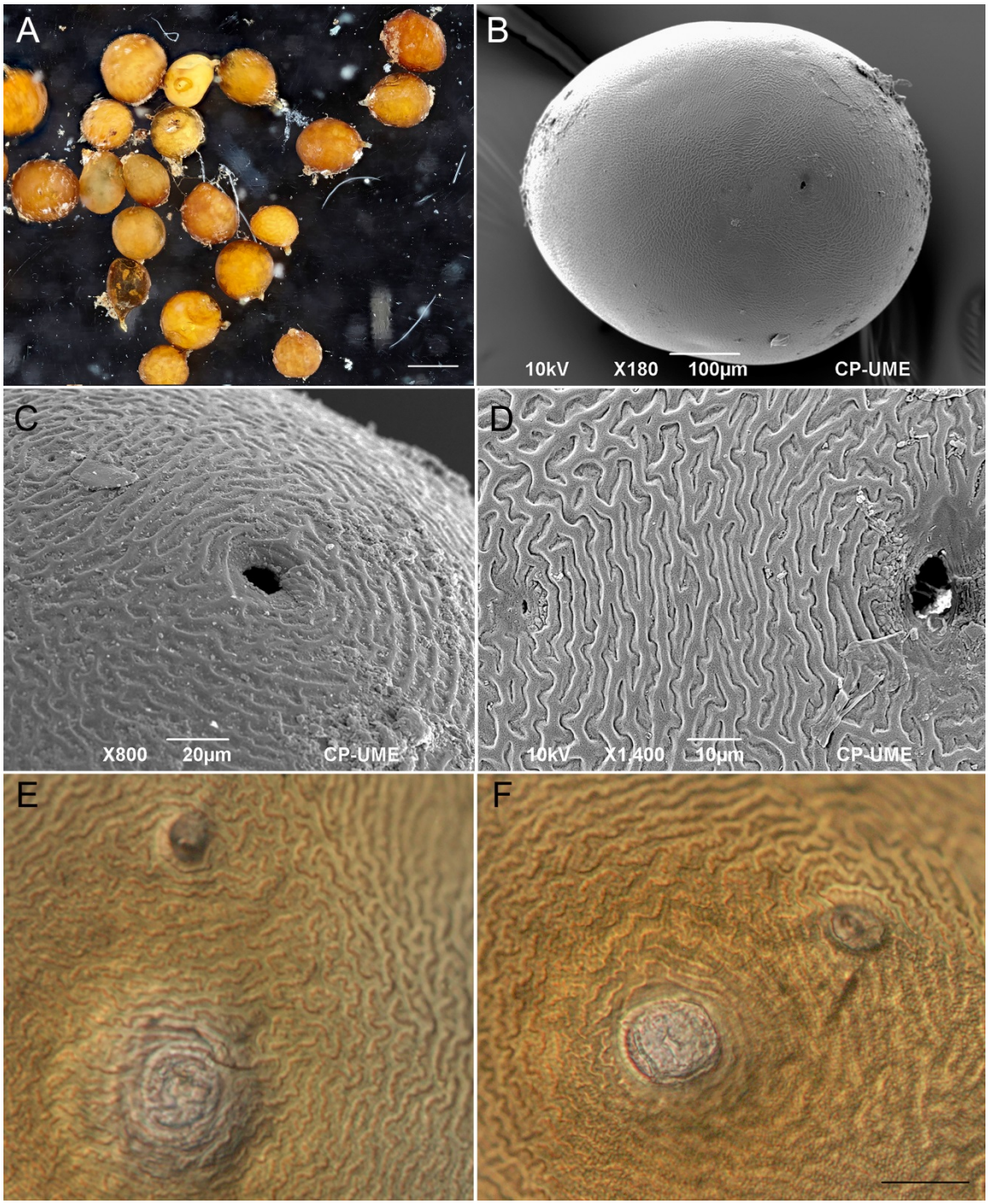

Fig. 2. Cysts of *Globodera mexicana*. A, B: Cysts; C-F: Vulval plates. Scale bars: A = 500 µm, E, F = 20 µm.

Cysts. Spherical in shape, full of embryonate eggs. Tan-brown colour with a lace-like pattern surface. Subcrystaline layer absent. Small tubercules present around the vulva. Vulva fenestra circumfenestrate, bullae absent. Anus as a small pore, located dorsally (Figs 1 & 2).

Second-stage juveniles. Cephalic region rounded, separated from the body by constriction and with four annuli, knobs anteriorly rounded or flattened. Pharyngeal glands fill the body cavity, overlapping intestine. Primordium slightly posterior to the mid-body; tail conical with pointed terminus (Fig. 3).

Males. Body ventrally arcuate following heat relaxation, approximately 1.0 mm long. Lip region rounded offset by a slight constriction. Metacorpus poorly developed and pharyngeal glands lobe shape,

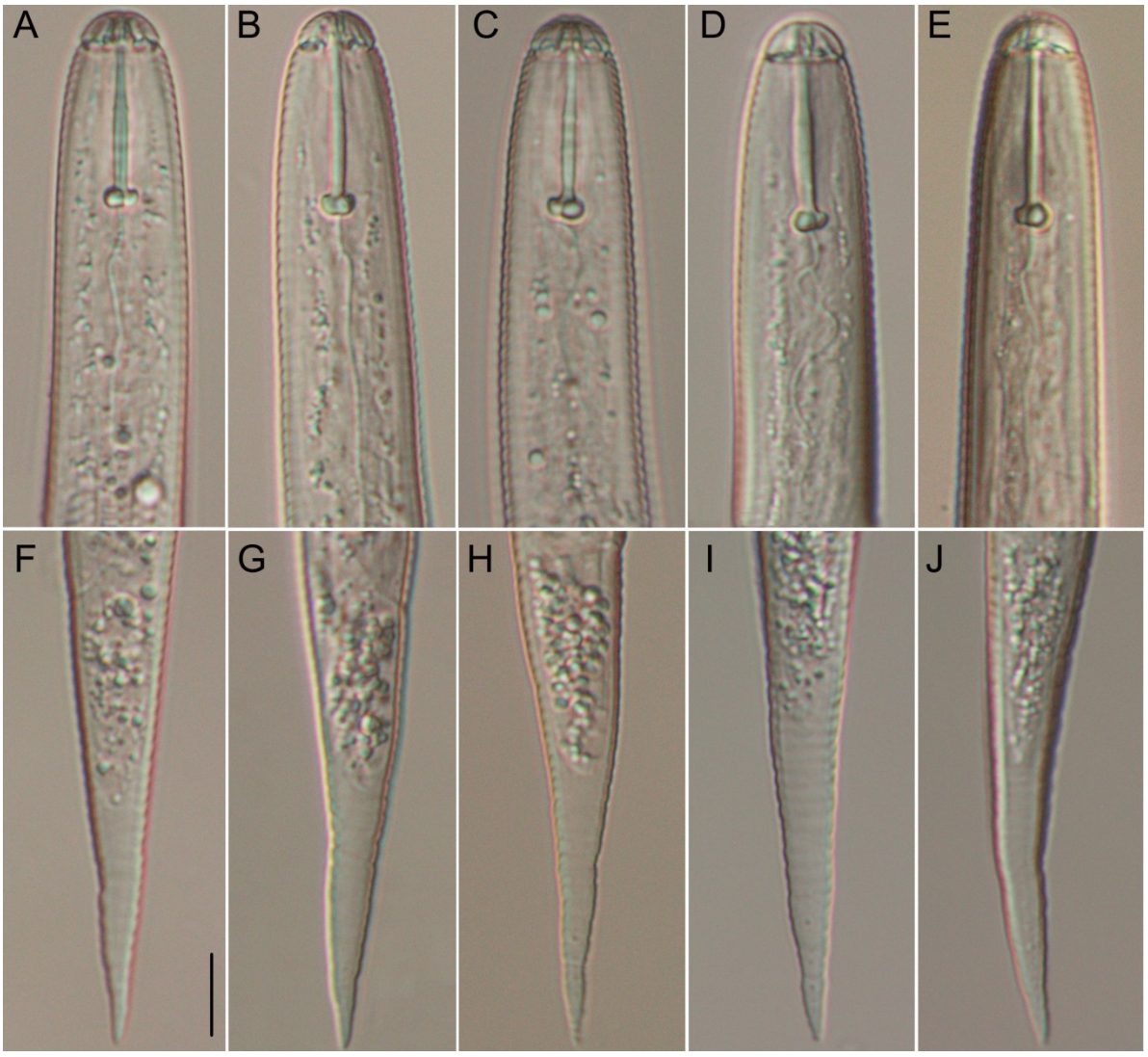

Fig. 3. Second-stage juveniles of *Globodera mexicana*. A-E: Anterior end; F-J: posterior end. Scale bars = 10 µm.

not occupying the width of body. Body annuli evident along the body and lateral field with four incisures. Tail very short.

Molecular characterisation of *Globodera mexicana*. Samples from different sources were used for molecular study (Table 1). Cysts of *G. mexicana* (CD3563, CD3564 and CD3565) were from three soil samples, whereas cysts of *G. mexicana* (CD3602) were from a glass vial under the name 'Mexican sample 178' in Stone's nematode collection. Additionally, three *G. mexicana* samples (CD2809, CD2814, CD2821) were used for amplification of *hsp90* gene. Sequences of these samples were compared with those already published.

Phylogenetic and sequence analysis with *COI* gene. A total of 31 sequences of *G. mexicana* were analysed. Fifteen new *COI* gene sequences for this species were obtained in this study. The alignment length was 443 bp. The haplotype network is given in Figure 4A with eight haplotypes revealed. One new haplotype, GmexCOI8, was identified in cysts from Stone's nematode collection. Maximal intraspecific *COI* gene sequence diversity for *G. mexicana* was 9.9%.

Phylogenetic and sequence analysis with *cytb* gene. A total of six sequences of *G. mexicana* were analysed. Three new *cytb* gene sequences for this species were obtained in this study. The alignment length was 516 bp. The haplotype network revealed six haplotypes (Fig. 4B). Three new haplotypes were designated in the present study for this species. Maximal intraspecific *cytb* gene sequence diversity for *G. mexicana* was 3.5%.

Phylogenetic and sequence analysis with ITS rRNA gene. A total of 27 sequences including two

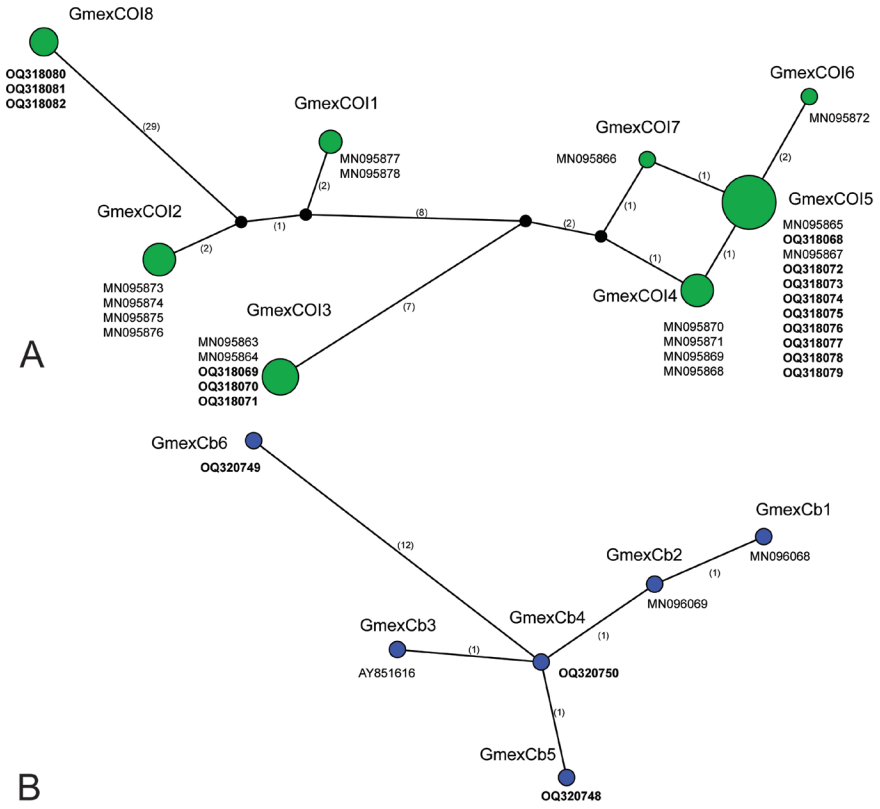

Fig. 4. Statistical parsimony networks showing the phylogenetic relationships between *COI* (A) and *cytb* (B) haplotypes of *G. mexicana*. Small black circles represent missing haplotypes. Pie chart sizes are proportional to the number of samples with a particular haplotype. Nucleotide changes between haplotypes are given for appropriate branches. New sequences are indicated by bold letters.

new sequences of *G. mexicana* were analysed. Phylogenetic relationships between *G. mexicana* and other *Globodera* parasitising solanaceous plants as inferred from analysis of the ITS rRNA gene sequence alignment are given in Figure 5A. Sequences of *G. mexicana* formed a distinct cluster. Maximal intraspecific ITS rRNA gene sequence diversity for *G. mexicana* was 0.6%.

Phylogenetic and sequence analysis with *hsp90* gene. A total of 22 sequences including six new sequences of *G. mexicana* were analysed. Phylogenetic relationships between *G. pallida* and *G. mexicana* parasitising solanaceous plants as inferred from analysis of the *hsp90* gene sequence alignment are given in Figure 5B. *Globodera mexicana* occupied basal positions at this tree. Maximal intraspecific *hsp90* gene sequence diversity for *G. mexicana* was 0.7%.

DISCUSSION

The results obtained in this study extended known morphometric variations for all stages of *G. mexicana*. Comparative analysis of morphometrics of *G. mexicana* and *G. pallida* does not allow clearly differentiating these species from each other using morphological and morphometrical characters. Several molecular markers, sequences of ITS rRNA, *hsp90*, *COI* and *cytb* genes can distinguish these species.

The analysis of *COI*, *cytb*, ITS rRNA and *hsp90* gene sequences suggested that *G. pallida* and *G. mexicana* are sister species with a divergence date of 1.6 million years ago (Subbotin *et al.*, 2020). It has been proposed that *G. mexicana* originated and diversified in Mexico. After adding a new sample from the Stone's nematode collection from unknown Mexican location in the analysis, maximal intraspecific *COI* and *cytb* gene sequence diversities for *G. mexicana* were estimated as 9.9% and 3.5%, respectively, compared with 4.7% and 0.8% obtained in a previous study (Subbotin *et al.*, 2020). The present study confirmed that the genetic diversity of *G. mexicana* is associated with zones of local topographical complexity. Mexico is crossed by large mountain systems (Sierra Madre Oriental,

Sierra Madre Occidental, Sierra Madre del Sur, Sierra de Chiapas, and the Trans-Mexican Volcanic Belt) corresponding to different geological provinces that differ vastly in age (Sosa *et al.*, 2018). These mountains are hotspots of biodiversity and endemism as a result of local and regional extinction, long-distance colonisation, and local recruitment (Subbotin *et al.*, 2020).

Considering our current knowledge on findings of *G. mexicana*, we can suggest that the cysts of its ancestor have dispersed to Mexico by passing through the northern Andes and Central America.

Table 3. Some morphological and morphometric characters used for diagnostics of five *Globodera* parasitising solanaceous plants (measurements are given in μm) after Subbotin *et al.* (2010), Handoo *et al.* (2012) and others.

Stage	Cysts		Second-stage juveniles			
Character / Species	Number of cuticular ridges between fenestra and anus	Granek's ratio	Body length	Stylet	Hyaline part of tail	Tail
G. mexicana	7-14	0.8-3.2	318-587	18-27	20-41	34-74
G. pallida	7-26	1.0-8.5	380-533	20-26	11-28	31-59
G. rostochiensis	12-31	1.3-9.5	425-505	19-24	20-27	44-51
G. ellingtonae	8-25	0.9-5.9	365-533	19-24	18-33	39-56
G. tabacum	5-15	1.0-4.2	410-527	19-28	21-28	50-56

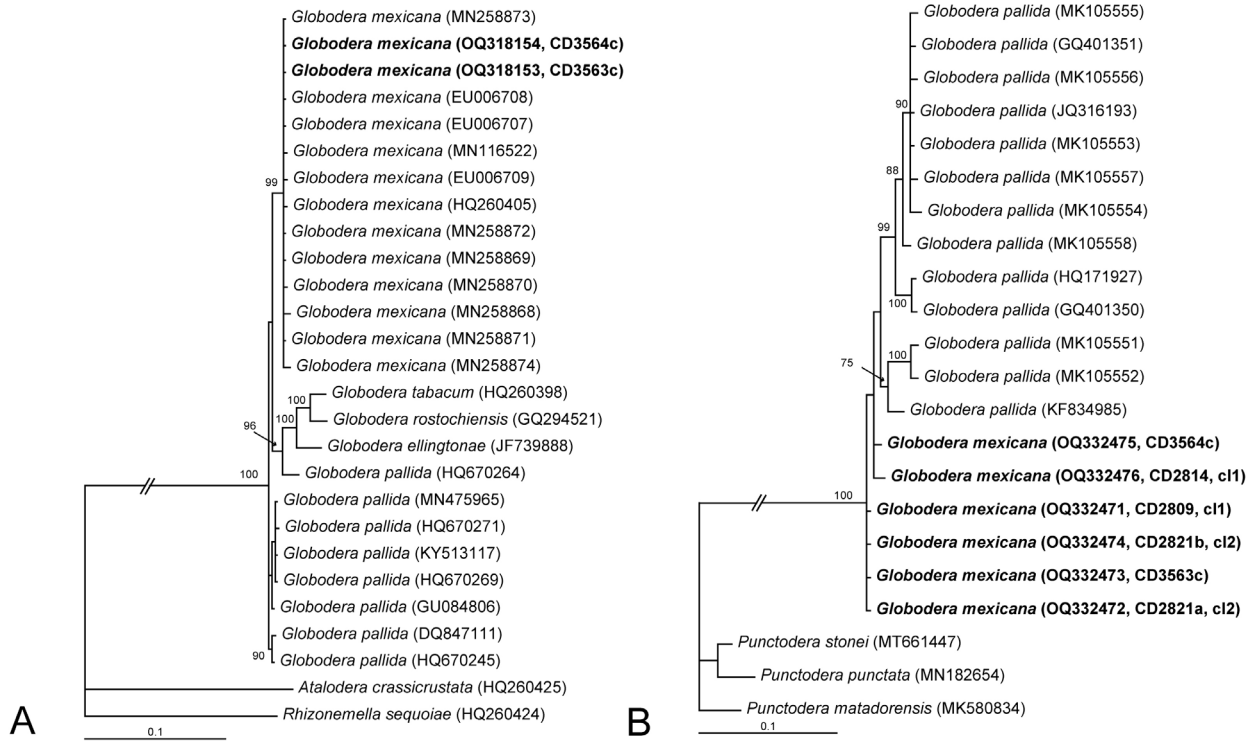

Fig. 5. Phylogenetic relationships of *Globodera mexicana* with some *Globodera* species parasitising solanaceous plants: Bayesian 50% majority rule consensus trees from two runs as inferred from analysis of the ITS rRNA (A) and the *hsp90* (B) gene sequence alignments under the GTR + I + G model. Posterior probabilities more than 70% are given for appropriate clades. New sequences are indicated by bold letters.

Future studies of the genus *Globodera* should focus on increased sampling in these regions of America from wild solanaceous plants to reconstruct more complete picture of phylogeography and evolution of this genus.

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REFERENCES

BANDELT, H., FORSTER, P. & RÖHL, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37-48. DOI: 10.1093/oxfordjournals.molbev.a026036

BLANCHARD, A., ESQUIBET, M., FOUVILLE, D. & GRENIER, E. 2005. Ranbmp homologue genes characterized in the cyst nematodes *Globodera pallida* and *Globodera 'mexicana'*. *Physiological and Molecular Plant Pathology* 67: 15-22. DOI: 10.1016/j.pmpp.2005.09.001

BOSSIS, M. & MUGNIÉRY, D. 1993. Specific status of six *Globodera* parasites of solanaceous plants studied by means of two-dimensional gel electrophoresis with a comparison of gel patterns by a computed system. *Fundamental and Applied Nematology* 16: 47-56.

CAMPOS-VELA, A. 1967. *Taxonomy, life cycle and host range of Heterodera mexicana n. sp. (Nematoda: Heteroderidae).* Ph.D. Thesis, University of Wisconsin, USA, 70 pp.

CHENNA, R., SUGAWARA, H., KOIKE, T., LOPEZ, R. GIBSON, T.J., HIGGINS, D.G. & THOMPSON, J.D. 2003. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research* 31: 3497-3500. DOI: 10.1093/nar/gkg500

CID DEL PRADO VERA, I. & SUBBOTIN, S.A. 2012. *Belonolaimus maluceroi* sp. n. (Tylenchida: Belonolaimidae) from a tropical forest in Mexico and key to the species of *Belonolaimus*. *Nematropica* 42: 201-210.

FENWICK, D.W. 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology* 18: 155-172.

FRANCO, N.F., I. CID DEL PRADO VERA, I. & LAMOTHE-ARGUMEDO, R. 2000. *Globodera bravoae* sp. n. (Tylenchida: Heteroderidae) from Mexico. *International Journal of Nematology* 10: 169-176.

GOLDEN, A. M. & ELLINGTON, D.M.S. 1972. Redescription of *Heterodera rostochiensis* (Nematoda: Heteroderidae) with a key and notes on closely related species. *Proceedings of the Helminthological Society of Washington* 39: 64-77.

GRENIER, E., BLOK, V.C., JONES, J.T., FOUVILLE, D. & MUGNIERY, D. 2002. Identification of gene expression differences between *Globodera pallida* and *G. mexicana* by suppressive subtractive hybridization. *Molecular Plant Pathology* 3: 217-226. DOI: 10.1046/j.1364-3703.2002.00111.x

HANDOO, Z.A, CARTA, L.K. SKANTAR, A.M. & CHITWOOD, D.J. 2012. Description of *Globodera ellingtonae* n. sp. (Nematoda: Heteroderidae) from Oregon. *Journal of Nematology* 44: 40-57.

HELDER, J., MOOIJMAN, P J W., ELSEN, S.J.J., VAN DEN MEGEN, H.H.B., VERVOORT, M.T.W., QUIST, C.W., BERT, W., KAREGAR, A., KARSSEN, G. & DECREAMER, W. 2014. Biological and systematic implications of phylogenetic analysis of ~ 2,800 full length small subunit ribosomal DNA sequences. *Proceedings of the 6th International Congress of Nematology,* Cape Town, South Africa: 26.

MUGNIERY, D., BOSSIS, M., & PIERRE, J.-S. 1992. Hybridations entre *Globodera rostochiensis* (Wollenweber), *G. pallida* (Stone), *G. virginiae* (Miller and Gray), *G. solanacearum* (Miller and Gray) et *G. 'mexicana'* (Campos-Vela). Description et devenir des hybrides. *Fundamental and Applied Nematology* 15: 375-382.

PICARD, D., SEMPERE, T. & PLANTARD, O. 2007. A northward colonisation of the Andes by the potato cyst nematode during geological times suggests multiple host-shifts from wild to cultivated potatoes. *Molecular Phylogenetics and Evolution* 42: 308-316. DOI: 10.1016/j.ympev.2006.06.018

PICARD, D., SEMPERE, T. & PLANTARD, O. 2008. Direction and timing of uplift propagation in the Peruvian Andes deduced from molecular phylogenetics of highland biotaxa. *Earth and Planetary Science Letters* 271: 326-336. DOI: 10.1016/j.epsl.2008.04.024

POSADA, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256. DOI: 10.1093/molbev/msn083

RONQUIST, F. & HUELSENBECK, J. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574. DOI: 10.1093/ bioinformatics/btg180

SACCO, M.A., KOROPACKA, K., GRENIER, E., JAUBERT, M.J., BLANCHARD, A., GOVERSE, A., SMANT, G. & MOFFETT, P. 2009. The cyst nematode SPRYSEC protein RBP-1 elicits Gpa2- and RanGAP2-dependent plant cell death. *PLOS Pathogens* 5(8): E1000564. DOI: 10.1371/journal.ppat.1000564

SEINHORST, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica* 4: 67-69. DOI: 10.1163/ 187529259X00381

SOSA, V., ARTURO DE-NOVA, J. & VÁSQUEZ-CRUZ, M. 2018. Evolutionary history of the flora of Mexico: Dry forests cradles and museums of endemism. *Journal of Systematics and Evolution* 56: 523-536. DOI: 10.1111/jse.12416

STARE, B.G, FOUVILLE, D., SIRCA, S., GALLOT, A., UREK, G. & GRENIER, E. 2011. Molecular variability and evolution of the pectate lyase (*pel-2*) parasitism gene in cyst nematodes parasitizing different solanaceous plants. *Journal of Molecular Evolution* 72: 169-181. DOI: 10.1007/s00239-010-9413-4

SUBBOTIN, S.A., MUNDO-OCAMPO, M. & BALDWIN, J.G. 2010. Systematics of cyst nematodes (Nematoda: Heteroderinae). In: Nematology Monographs and Perspectives, Volume 8A (D.J. Hunt & R.N. Perry Eds). 512 pp. Leiden, The Netherlands, Brill.

SUBBOTIN, S.A., CID DEL PRADO, I., MUNDO-OCAMPO, M. & BALDWIN, J.G. 2011. Identification, phylogeny and phylogeography of circumfenestrate cyst nematodes (Nematoda: Heteroderidae) as inferred from analysis of ITS-rDNA. *Nematology* 13: 805-824. DOI: 10.1163/138855410X552661

SUBBOTIN, S.A., FRANCO, J., KNOETZE, R., ROUBTSOVA, T.V., BOSTOCK, R.M. & CID DEL PRADO VERA, I. 2020. DNA barcoding, phylogeny and phylogeography of the cyst nematode species from the genus *Globodera* (Tylenchida: Heteroderidae). *Nematology* 22: 269-297. DOI: 10.1163/15685411-00003305

THIÉRY, M., MUGNIÉRY, D., BOSSIS, M. & SOSA-MOSS, C. 1997. Résultats de croisements entre *Globodera pallida* Stone et *G. 'mexicana'* Campos-Vela: héritabilité du développement sur pomme de terre etnotion d'espèce. *Fundamentals of Applied Nematology* 20: 551-556.

URL: http:/popart.otago.ac.nz (accessed: December 5, 2022).

I. Cid del Prado Vera, J.A.M. Ceron, V. Orlando, R. Lawson, T. Prior and S.A. Subbotin. Морфологическая и молекулярная характеристика мексиканской цистообразующей нематоды, *Globodera mexicana* Subbotin, Mundo-Ocampo & Baldwin, 2010 (Tylenchida: Heteroderidae).

Резюме. Мексиканская цистообразующая нематода, *Globodera mexicana*, была обнаружена на диких пасленовых растениях, собранных в нескольких местах Мексики. Анализ последовательностей генов *COI*, *cytb*, ITS рРНК и *hsp90* позволил предположить, что *Globodera pallida* и *G. mexicana* являются сестринскими видами. В этом исследовании было предоставлено 15 новых *COI*, три новых c*ytb*, шесть новых *hsp90* и две новые ITS рРНК последовательности генов. Максимальное внутривидовое различие в последовательностях гена *COI* для *G. mexicana* оценивается в 9,9%. Дается морфологическое описание *G. mexicana* из типового места обитания и других районов. Принимая во внимание наши знания о находках *G. mexicana*, было высказано предположение, что этот вид возник и диверсифицировался в Мексике, а цисты его предка проникли в Мексику, миновав северные Анды и Центральную Америку. Будущие исследования по изучению биоразнообразия рода *Globodera* должны быть сосредоточены на увеличении количества сборов в этих регионах Центральной и Южной Америки с диких пасленовых растений. Это позволит воссоздать более полную картину филогеографии и эволюции этого рода.