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Ribosomal DNA Comparisons of *Globodera* from Two Continents[1]

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Abstract: Ribosomal DNA (rDNA) sequence data were compared for five species of *Globodera*, including *G. rostochiensis*, *G. pallida*, *G. virginiae*, and two undescribed *Globodera* isolates from Mexico collected from weed species and maintained on *Solanum dulcamara*. The rDNA comparisons included both internal transcribed spacers (ITS1 and ITS2), the 5.8S rRNA gene, and small portions of the 3′ end of the 18S gene and the 5′ end of the 28S gene. Phylogenetic analysis of the rDNA sequence data indicated that the two potato cyst nematodes, *G. pallida* and especially *G. rostochiensis*, are closely related to the Mexican isolates, whereas *G. virginiae* is relatively dissimilar to the others and more distantly related. The data are consistent with the thesis that Mexico is the center of origin for the potato cyst nematodes.

Key words: *Globodera*, *G. rostochiensis*, *G. pallida*, *G. virginiae*, *G. tabacum*, nematode, ribosomal DNA, rDNA ITS1 and ITS2, 5.8S rRNA gene.

Species of *Globodera* are known from many parts of the world. In North America, three apparently indigenous species have been described from the eastern United States. The species, which are morphologically similar, are *G. virginiae* (Miller and Gray, 1968) Behrens, 1975; *G. solanacearum* (Miller and Gray, 1972) Behrens, 1975; and *G. tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975. Stone (37) considered *G. virginiae* and *G. solanacearum* to be subspecies of *G. tabacum*, as did Bossis and Mugniéry (5). Baldwin and Mundo-Ocampo (4) termed this the *G. tabacum* complex. A number of undescribed species have been collected in Mexico by one of us (LIM) and studied over the years (4,15,28). In addition to these North American species, *Globodera* species have been described in other parts of the world, but the most widely studied of these species are the two potato cyst nematodes, *G. rostochiensis* (Wollenweber, 1923) Behrens, 1975 and *G. pallida* (Stone, 1973) Behrens, 1975. Both species are thought to have originated in the mountains of South America and been carried elsewhere along with their potato host (4).

Until recently, *Globodera* species were distinguished on the basis of morphological differences and their ability to reproduce on various hosts. Sorting the species on the basis of these characteristics has proved difficult, however, and some of the diagnostic characters (e.g., patterns in the anal-vulval region and stylet characteristics) may overlap among various populations of the different species (4). Various biochemical tests have been devised recently to separate the two potato cyst nematodes (3,7,33,36,38).

We have included five *Globodera* isolates in our ongoing study of ribosomal DNA (rDNA) in cyst nematodes, viz., *G. rostochiensis*, *G. pallida*, *G. virginiae*, and two undescribed, but well-characterized, isolates of *Globodera* from Mexico that may be distinct species. Our rDNA data include the complete nucleotide sequence of both internal transcribed spacer regions (ITS1 and ITS2) and the 5.8S rRNA gene between them. Our goal was to determine whether these rDNA data could be useful for phylogenetic analysis. In addition, we present examples of protein patterns of *Globodera* obtained by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), and several morphological comparisons of juveniles and cysts.

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Materials and Methods

Sources of isolates: Globodera rostochiensis, Feltwell population, originally from Cambridgeshire, England, and *G. pallida,* Cadishead population, originally from Lancashire, England, were supplied by Dr. Alan Stone at Rothamsted in 1985. Young female nematodes, from stock cultures in the Rothamsted glasshouse, were picked from roots, rinsed in tap water, and divided into tubes of 30 nematodes each. The nematodes were killed by freezing in 0.2 M sodium borate buffer and transported by airplane on dry ice to the Purdue University laboratory, where they were stored frozen at −20 C or in liquid nitrogen until used for DNA or protein samples. Young female nematodes of *Globodera virginiae* (29), originally from the type locality in Virginia (U.S.A.) and maintained in stock cultures by LIM, were selected, rinsed, and frozen in borate buffer until used. Both *Globodera* isolates from Mexico were from stock cultures maintained on *Solanum dulcamara* by LIM and were collected and frozen until used as described above. In 1975, Drs. C. Sosa-Moss, A. R. Stone, and LIM collected the isolate X-140 from a sparsely wooded area on the weed *Physalis orizabe* at Santa Ana near Juchitepec in the Estado de México. Isolate X-76 is from a culture derived from a single cyst collected from the roots of the weed *Solanum elaeagnifolium* in 1975 by LIM at La Colorado in the Estado de Coahuila.

rDNA amplification, cloning, and sequencing: To prepare the nematodes for rDNA analysis, we used a method adapted from Caswell-Chen et al. (9), for which we substituted Instagene (BioRad) for the Chelex preparation. One or two frozen female nematodes were crushed in 20 µl cold TE buffer, using a Radnoti (Thomas Scientific, Swedesboro, NJ) 25-µl-size glass homogenizer, with the homogenate transferred to a 0.5-ml tube and either used immediately or stored at −20 C. Five such preparations were made for each isolate. Prior to amplification by polymerase chain reaction (PCR), the homogenate was spun in the microfuge for 3 minutes at 14,000 RPM (= 16,000*g*) and the supernatant discarded. Sixty µl Instagene was added to the pellet and the procedure completed according to the manufacturer's protocols. We used 10 µl of a 1:10 dilution of the preparation for each 25 µl PCR reaction. Other methods were essentially as previously described (19,20). Standard PCR (35) was used with reagents from Perkin Elmer (Norwalk, CT) and Promega (Madison, WI) and a COY Tempcycler model 50. Primers for PCR amplification were as described in a previous paper (19). The amplified DNA for these *Globodera* isolates was slightly less than 1 Kb in length and spanned the two ITS regions, including the 5.8S gene (Fig. 1).

Amplified rDNA was cloned into the TA pCR 1000 cloning vector of Invitrogen (San Diego, CA) as described in a previous paper (19,20). Double-stranded sequencing was performed using Sequenase version 2.0 from U.S. Biochemical (Cleveland, OH). We sequenced from two or more clones of each isolate using primers specified for the vector as described (19,20); in some cases, we also sequenced directly from the amplified DNA product. For the latter procedure, we first concentrated the DNA by precipitation using linear polyacrylamide as a carrier (23) and purified it by electrophoresis followed by treatment with Geneclean (Bio 101). The DNA was annealed to one or the other of the PCR primers in a mixture of Sequenase (USB) reaction buffer plus 5% Nonidet P-40 followed by boiling 5 minutes and a plunge into liquid nitrogen (1). After slowly warming the DNA to room temperature, we proceeded with the Sequenase protocols.

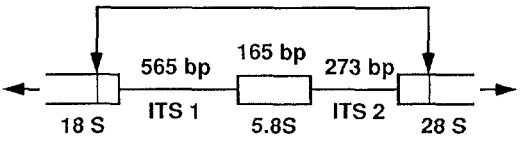

FIG. 1. Diagram with arrows indicating amplified region of rDNA, with base pair (bp) numbers based on Mexican isolate X-140. ITS = internal transcribed spacer. 18S, 5.8S, and 28S are rDNA genes.

In order to sequence the entire amplified region in all clones, additional primers were designed based on comparative study of internal sequence. These primers were as follows: for *G. rostochiensis,* the forward internal sequencing primer was 5′-ATGGTGAGCCGACGATTGC-3′; for the rest of the isolates, the forward internal sequencing primer was 5′-CGTCTGTGCGTCGTTGAGC-3′. The reverse internal sequencing primer was 5′-ACTCCAATGGCGCAATGTG-3′ for all isolates. Sequence data were from multiple clones and both strands. Each sequencing run for a given clone was repeated three to six times to ensure accuracy of the sequence.

DNA sequence comparisons, alignment, and phylogenetic analysis: Sequence data for each isolate were aligned with each other using the computer program GAP in the Sequence Analysis Software Package of the Genetics Computer Group (10). GAP uses the algorithm of Needleman and Wunsch (30) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps, and also calculates pairwise similarity. Gap weight was varied to test the stability of the alignment (40). Multiple alignment was done initially using the PILEUP program in the same computer package, followed by manual adjustments to improve the fit (40). The phylogenetic analysis was performed on the aligned data using the computer program PAUP (39), which infers phylogenies from discrete-character data under the principle of maximum parsimony. PAUP finds the tree (also called a cladogram) that minimizes the amount of evolutionary change needed to explain the available data under a prespecified set of constraints. Bootstrap analysis (11), also included in the PAUP package, was performed to establish confidence limits.

2-D PAGE protein gels: O'Farrell's (31) methods were modified as described earlier (14,20). Briefly, the nematodes for protein analysis were homogenized over ice in a 0.2 M sodium borate buffer at pH 9, with a ground-glass homogenizer. The homogenate was centrifuged at 12,800*g* for 5 minutes and the supernatant dialyzed against the borate buffer and then stored over liquid nitrogen. Proteins were labeled in vitro by reductive methylation with formaldehyde and sodium (^{3}H) borohydride (26). Urea sample buffer, which contained 9.5 M urea, 2% (v/v) Nonidet P-40, and 5% (v/v) β-mercaptoethanol, was added to each labeled, precipitated, and washed (with acetone-ether 1:3 v/v) protein sample, and the sample was stored at −80 C.

Proteins in a 25- to 50-µl sample were separated by isoelectric focusing across a pH range of 4.0 to 5.6 in a tube gel using a Bio-Rad model 155-gel electrophoresis cell. The anode electrode solution was 0.01 M H_3PO_4, and the cathode electrode solution was 0.02 M NaOH. Electrophoresis was carried out at 400 V for 18 hours, and the power was then increased to 800 V for 1 hour to focus the proteins. In the second dimension, each tube gel was placed on top of a sodium dodecyl sulfate 12% (w/v) polyacrylamide slab gel, 1.2 mm thick. Electrophoresis was in a Bio-Rad Protean dual vertical slab gel electrophoresis cell at 20 mA per gel for approximately 5 hours. Molecular weight standards (Bethesda Research Laboratories, Gaithersburg, MD) were run in the second dimension, with the nematode proteins. Labeled proteins were located on the gels by fluorography with the EN^{3}HANCE procedure (New England Nuclear Research Products, Boston, MA). At least 10 gel patterns were obtained for each isolate, and proteins from each isolate were run in both dimensions in the same electrophoresis cell with proteins from other isolates to permit tracing small variations in protein positions. Several autoradiographs were made from each gel at a range of exposure times. The transparent autoradiographs were overlaid and compared directly. As is often done with 2-D PAGE gels, we used internal "landmark" spots to align gels for comparison (6,34,41). Proteins with identical electrophoretic properties were assumed to be

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      1                                                            50
X14   CGTAACAAGG TAGCTGTAGG TGAACCTGCT GCTGGATCAT TACCCAAGTG
X76   .......... .......... .......... .......... ..........
ROS   .......... .......... .......... .......... ..........
PAL   .......... .......... .......... .......... ..........
VIR   .......... .......... .......... .......... ..........

      51                                                           100
X14   ATACCAATTC ACCATCTACC TGCTGTCCAG TTGAGTCAGT GTGGGCAACA
X76   .......... .......... .......... .......... ..........
ROS   .......... ....C..... .......... .......... ..........
PAL   .......... ....C..... ......T... .......... ..........
VIR   .....T.... ....C..... .........C. ...T..T.A. ..........

      101                                                          150
X14   CCACATGCCT CCGTTTGTTG TTGACGGACA CATGCCCGCT GTGTATGGGC
X76   .......... .......... .......... .......... ..........
ROS   .......... .......... .......... .......... ..........
PAL   .......... .......... .......... .......... A...T.....
VIR   .......... .......C.. C......... ..GA...TAC .-.AGA....

      151                                                          200
X14   TGGCACATTG ACCAACAGTG TACGGACAGC GCCCTGTGCG CATGAGTGTT
X76   .......... .......... .......... .......... ..........
ROS   .......... .......A.. .......... .......... ..........
PAL   .......... .T.....A.. ..T....... .......... ..........
VIR   .......... .......C.. A......G.. .G..CCGTG. ..C....-..

      201                                                          250
X14   GGGGTGTAAC CGATGTTGGT GGCCCTATGG -TGAGCCGAC GATTGCTGCT
X76   .......... .......... .......... -.......... ..........
ROS   .......... .......... .......... -.......... ..........
PAL   .......... .......... .....A.... -...CT.... .........T.
VIR   ......CT.. T..A...... ..T..A.... G........-- .-.......-C

      251                                                          300
X14   ATCGTCGGGT CGCTGCACCA ACGGAGGAAG CACGCCCACAG GGCACCCTA
X76   .......... .......... .......... ..........  .........
ROS   G......... .......... .......... ..........  .........
PAL   G......... .......... .......TG. ..........  .........
VIR   GC......C. .A........ .......TG. .....T..... ..GCA..C.

      301                                                          350
X14   ACGGCTGTGC TGGCGTCTGT GCGTCGTTGA GCGGTTGTTG CGCCTTGCGC
X76   .......... .......... .......... .......... ..........
ROS   ......A... .......... ..--...... .......... .....C..A-
PAL   .......... .......... .......... .......... .--.......
VIR   .......... .......... .......... .......... .........TG
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FIG. 2. Alignment of 42 nucleotide bases of 18S rRNA gene (italics), plus rDNA ITS1 sequence for Mexican isolate X-140 (X14), Mexican isolate X-76 (X76), *G. rostochiensis* (ROS), *G. pallida* (PAL), and *G. virginiae* (VIR). All base notations are for the nontranscribed strand. Numbering is based on sequence for X14, and the isolates are listed in order of increasing dissimilarity to X14. Sequence differences are uncorrected for multiple changes at a site.

identical (2,6,31). A spot consistently pale on patterns of one isolate and dark on patterns of another isolate was considered to be present in both isolates, and analysis was limited to spots reproducible in all gel patterns of a given isolate.

Morphological data: All morphological data were collected by established methods in the laboratory of LIM, as part of a large study of variability in *Globodera* (unpubl.). The data reported here for second-stage juvenile nematodes include stylet length, stylet knob width in lateral view, and distance between median bulb and the ventral pore. Data for cysts include calculations of Granek's ratios and the ratio between length (without neck) and radial width of the anterior half of young brown cysts re-

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     351                                                        400
X14  G-ATATGCTA ACATGG-AGT GTATGCTGCT ATTCCATGTT GTACGTGCCG
X76  .-........ ......-... .......... .......... ..........
ROS  .-........ ......-... ...-...... .C........ ..........
PAL  .G.......G .......-.A. ...G...... .......... ..........
VIR  .-.C..A..G ..G...A..G ..GGT.GTAC C....TC..C ....CG.TG.

     401                                                        450
X14  TACCCCGCGG CATATCTGCG CTTGTGTGCT ACGTCCGTGG CCGTGATGAG
X76  .......... .......... .......... .......... ..........
ROS  ....TT.... ...G...... .......... .......... ..........
PAL  ....-..... ...G...... .......... .........A ..........
VIR  .....A.... T..G....T. .......... .........A ..........

     451                                                        500
X14  ACGACGTGTT AGGACCCGTG CC-TGGCATT GGCACGTGGT TTAAGACTTG
X76  .......... .......... ..-....... .......... ..........
ROS  .......... .......... ..T....... .......... ..........
PAL  .......... .......... ..-....... .......... ..........
VIR  .......... .......... ..-....... ....T..... ........CA

     501                                                        550
X14  ATGA-TGCCC G-CAGCACGC CAGCTTTTTC TCATTTTTAT TTATTTTTTT
X76  ....G..... .-........ .......... .......... ..........
ROS  ....-..... .-........ .......... C......... ..........-
PAL  ....-..... .-........ .......... .......... AA.......A
VIR  ....G..... .A........ ...TG..... .......C.A .A........

     551          567
X14  ATGCAATTCG ATTGCTA
X76  .......... .......
ROS  .......... .......
PAL  ........T. .......
VIR  .C....CATT G.....-
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FIG. 2. Continued.

tained on a 300-μm-pore sieve. Specimens were from population WC3 for *G. rostochiensis* and COL C for *G. pallida*. The specimens used for X-140 and *G. virginiae* were from the same populations as those used for the biochemical analyses.

RESULTS

rDNA sequence data

Dissimilarity in ITS1, 5.8S, and ITS2: Figures 2–4 show the rDNA sequence for ITS1, 5.8S, and ITS2. The sequence includes 42 nucleotides of the 3′ end of the 18S gene and 59 nucleotides of the 5′ end of the 28S gene. The DNA sequence for ITS1, which begins at nucleotide 43 (Fig. 2), showed more differences among the five isolates than are found in ITS2, which ends at nucleotide 946 (Fig. 4). For both ITS regions, *G. virginiae* is the most dissimilar. Based on pairwise dissimilarities calculated before the multiple alignment of the sequences, *G. virginiae* was 16–17% dissimilar to the rest in ITS1 and 14–18% dissimilar in ITS2 (Tables 1, 2). *Globodera pallida* was 5% dissimilar to *G. rostochiensis* and the Mexican isolates in ITS1, whereas *G. rostochiensis* was 2% dissimilar to the Mexican isolates (Table 1). As might be expected, all five isolates were more similar in the 5.8S gene, with identical sequence found for all except *G. virginiae*, which was 4% dissimilar to the rest. Of particular interest is the base difference (A to G, nucleotide 617 in Fig. 3) in an area of the 5.8S gene that is highly conserved among the animal and plant species for which 5.8S sequence is published or in Genbank (unpubl.). In the conserved 5′ portion shown of the 28S gene (Fig. 4), the two differences (A to G, nucleotide 969, and C to T, nucleotide 970) are interesting, particularly as these differences also occur in the 28S sequence of the avenae group of *Heterodera* (20 and unpubl.). The overall pairwise similarities of Table 3, based on all sequences combined (Figs. 2–4), are those

calculated during the multiple alignment that formed the basis for the phylogenetic analysis. Overall dissimilarity between *G. virginiae* and the rest was 19–22% following multiple alignment of the five sets of sequence data. Overall dissimilarity between the potato cyst nematodes and the Mexican isolates was 5–6%.

Phylogenetic analysis: Using the exhaustive search option, the PAUP program evaluated 15 trees with lengths varying from 153 to 169. Only one tree was found with the minimum length; the next two shortest trees had a length of 160. In the minimum-length tree (Fig. 5), *G. rostochiensis* was closest to the two Mexican isolates, with *G. pallida* coming off a separate node. *Globodera virginiae* was designated outgroup to the rest for rooting the tree. A bootstrap analysis (Heuristic search with 100 replicates) indicated good support for the minimum length tree (Fig. 5).

Protein patterns

Overall, the 2-D PAGE protein patterns among these *Globodera* isolates appeared to share many features. Similarities in protein constellations (i.e., groups of protein/polypeptide spots) can be observed by inspecting the examples in Fig. 6, even in the absence of detailed quantitative analysis. Of special interest is the apparent overall similarity between the pattern for *G. rostochiensis* and that of the Mexican isolate X-140 (Fig. 6A,B). An example of one constellation of polypeptides nearly identical in all replicates of the two patterns is indicated by arrows. The protein pattern for *G. virginiae* (Fig. 6C) has similarities to other *Globodera* isolates. For comparison, Fig. 6D shows the 2-D protein pattern for another *Globodera* species, *G. tabacum*, also originally collected from the eastern United States (Connecticut). The protein patterns for *G. virginiae* and *G. tabacum* are similar but not identical (Fig. 6C,D). Arrows indicate one of the several constellations of small protein spots that we found to differ consistently between these two species. We have earlier reported on similarities-differences in the patterns of *G. rostochiensis* and *G. pallida* (18).

```
     568                                                        617
X14  AAATATTCTA GTCTTATCGG TGGATCACTC GGCTCGTGGA TCGATGAAGA
X76  .......... .......... .......... .......... ..........
ROS  .......... .......... .......... .......... ..........
PAL  .......... .......... .......... .......... ..........
VIR  .......... .......... .......... .......... .........G

     618                                                        667
X14  ACGCAGCCAA CTGCGATAAT TAGTGTGAAC TGCAGAAACC TTGAACACAG
X76  .......... .......... .......... .......... ..........
ROS  .......... .......... .......... .......... ..........
PAL  .......... .......... .......... .......... ..........
VIR  .......... ........C. .......... .........T ..........

     668                                                        717
X14  AACTTTCGAA TGCACATTGC GCCATTGGAG TGACATCCAT TGGCACGCCT
X76  .......... .......... .......... .......... ..........
ROS  .......... .......... .......... .......... ..........
PAL  .......... .......... .......... .......... ..........
VIR  .......... .......... .......... .A.T....T. ..........

     718          732
X14  GGTTCAGGGT CGTAA
X76  .......... .....
ROS  .......... .....
PAL  .......... .....
VIR  .......... .....
```

FIG. 3. Alignment of rRNA 5.8S gene sequences for Mexican isolate X-140 (X14), Mexican isolate X-76 (X76), *G. rostochiensis* (ROS), *G. pallida* (PAL), and *G. virginiae* (VIR). All base notations are for the nontranscribed strand. Numbering is based on sequence for X14, and the isolates are listed in order of increasing dissimilarity to X14. Sequence differences are uncorrected for multiple changes at a site.

```
     733                                                        782
X14  CCAAAAAATG CACTGCGTAT GCGTGTTTTA TTTGCTAAGA TCACGCTTCG
X76  .......... ......A... ..A....... .......... ..........
ROS  .......... ......A.G. .......... .......... .-........
PAL  .......... ......A.G. .......... .......... .-........
VIR  .......... ......A.G. .........G AC........ ......C.G.

     783                                                        832
X14  GTGTGTTCTT GCATTACCAT TGAATCGTAC GCTGTGTAGC GTTGGACGTC
X76  .......... ......C... .......... .......... ..........
ROS  .C........ ...-..CT.. .......... .......... ..........
PAL  .C........ ...-..C... .......... .........T ..........
VIR  TC........ ....A.--.A CT.TG..... .......G.. .........G

     833                                                        882
X14  GTGGCGCGAA AATGTGTTG- --TCATTCGC GCTTTACAGA CCGTAATTTA
X76  .......... .........- --........ .......... ..........
ROS  .......... .........- --........ .......... ..........
PAL  .......... .........- ---.T..... .......... ..........
VIR  C...T...G. .........T TC..T.C..T .......... ..........

     883                                                        932
X14  GGCACGCCCT TCGTTCACGT GCGATAGCTG AATGCCTCGC CAATAGGCAT
X76  .......... .......... .......... .......... ..........
ROS  .......... ........A. .......... .......... ..........
PAL  .......... ........A. .......... .......... ..........
VIR  ..T...T..C .......TA. .........A .....--..T ........G.

     933                                                        982
X14  TTGCAATTGA ACATTTCGAC CTGAACTCAG ACGTGAACAC CCGCTGAACT
X76  .......... .......... .......... .......... ..........
ROS  .......... .......... .......... .......... ..........
PAL  .......... .......... .......... .......... ..........
VIR  ...T.C.... CTGC...... .......... ......GT.. ..........

     983                      1005
X14  T.AGCATATC ATTTAGCGGA GGA
X76  .......... .......... ...
ROS  .......... .......... ...
PAL  .......... .......... ...
VIR  .......... .......... ...
```

FIG. 4. Alignment of rDNA ITS2 sequences plus 59 nucleotide bases of 28S rRNA gene (italics) for Mexican isolate X-140 (X14), Mexican isolate X-76 (X76), *G. rostochiensis* (ROS), *G. pallida* (PAL), and *G. virginiae* (VIR). All base notations are for the nontranscribed strand. Numbering is based on sequence for X14, and the isolates are listed in order of increasing dissimilarity to X14. Sequence differences are uncorrected for multiple changes at a site.

Morphological data

The data in Table 4 indicate that *G. rostochiensis* and X-140 are more similar to each other in mean stylet length, stylet knob width in lateral view, and distance from median bulb to ventral pore than they are to *G. pallida* and *G. virginiae*. The data in Table 5 show that mean values for Granek's ratio and the length-width ratio

TABLE 1. Pairwise percentage nucleotide dissimilarities in rDNA ITS1 for *Globodera* species (isolates) calculated prior to multiple alignment.

	X14	X76	ROS	PAL	VIR
X14	—	.00	.02	.05	.17
X76		—	.02	.05	.16
ROS			—	.05	.16
PAL				—	.17

TABLE 2. Pairwise nucleotide dissimilarities in rDNA ITS2 for *Globodera* species (isolates) calculated prior to multiple alignment.

	X14	X76	ROS	PAL	VIR
X14	—	.01	.02	.03	.18
X76		—	.02	.04	.16
ROS			—	.02	.14
PAL				—	.15

TABLE 3. Overall pairwise nucleotide dissimilarities following multiple alignment of all combined rDNA sequence (Figs. 2–5) for *Globodera* isolates.

	X14	X76	ROS	PAL	VIR
X14	—	.002	.05	.06	.19
X76		—	.05	.06	.19
ROS			—	.06	.22
PAL				—	.21

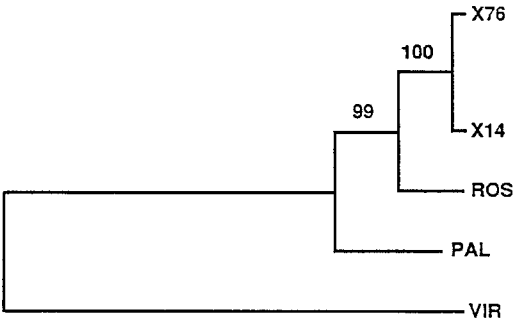

FIG. 5. The shortest tree, with total length = 153, based on all sequence data, Figs. 2–4, using exhaustive search option of PAUP. X76 = Mexican isolate X-76, X14 = Mexican isolate X-140, ROS = *G. rostochiensis*, PAL = *G. pallida*, VIR = *G. virginiae*. Rooting with VIR designated as outgroup. Branch lengths are drawn proportional to the number of inferred changes. Bootstrap values (11), based on 100 replications, are indicated on horizontal line segments. Overall consistency index = 0.994.

of cysts are similar for *G. rostochiensis* and X-140, and different from those of *G. pallida* and *G. virginiae*.

DISCUSSION

The high degree of DNA sequence similarity (= 1 − dissimilarity) between the potato cyst nematodes, especially *G. rostochiensis* and the two Mexican isolates of *Globodera*, was of interest, particularly when contrasted with the high degree of sequence dissimilarity between *G. virginiae* and the other four isolates. The similarity in 2-D protein patterns found for the Mexican isolate X-140 and *G. rostochiensis* supports the argument that the phylogenetic relationships based on rDNA truly reflect species trees and not gene trees. This argument is further supported by morpho-

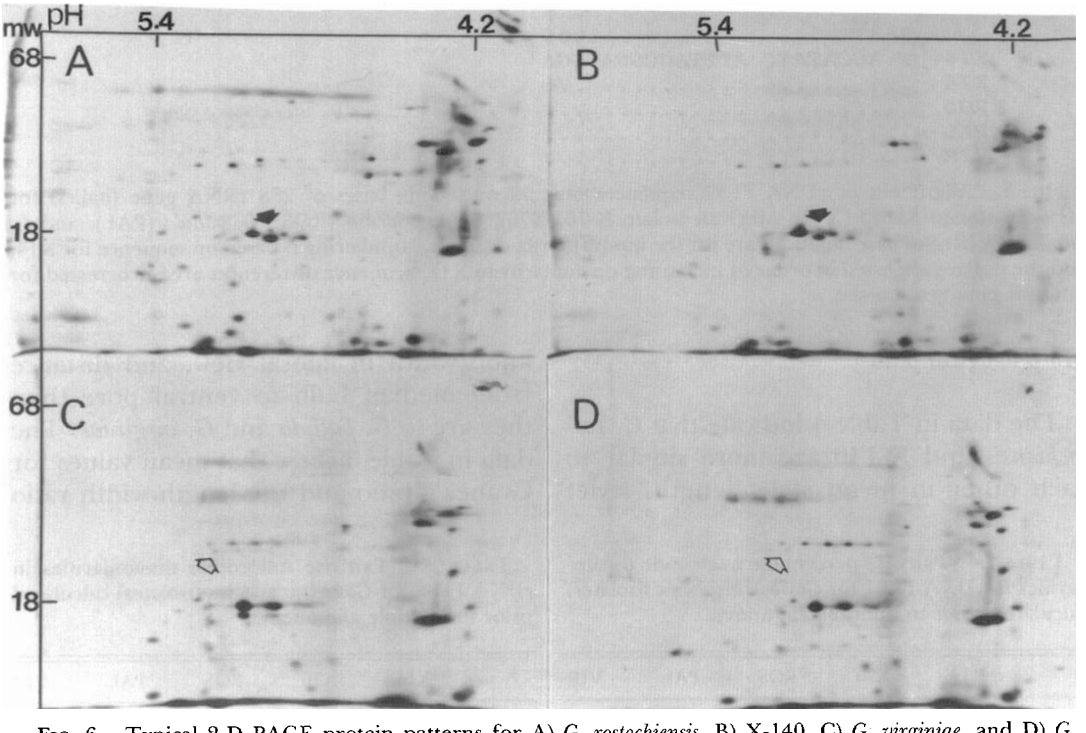

FIG. 6. Typical 2-D PAGE protein patterns for A) *G. rostochiensis*, B) X-140, C) *G. virginiae*, and D) *G. tabacum*. Arrows in A and B indicate a constellation of polypeptides that is nearly identical in all replicates of the two patterns. Arrows in C and D indicate a constellation of small protein spots that differs consistently in patterns of these two species. Molecular weights are given in thousands.

TABLE 4. Dimensions (μm) of second-stage juveniles of four isolates of *Globodera* species cultured on *Solanum dulcamara.*

Species (isolate)	Stylet length	Stylet knob width in lateral view	Median bulb to ventral pore
G. rostochiensis	22.5 a	3.8 a	40.0 a
X-140	23.0 a	3.8 a	38.2 a
G. pallida	23.9 b	4.7 b	45.3 b
G. virginiae	24.3 b	4.7 b	42.1 b

Data are for means of $n = 21$. Means followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's HSD test.

logical similarities between X-140 and *G. rostochiensis.* Neither of the two Mexican isolates studied here reproduces on *Solanum tuberosum,* but both can be maintained on other *Solanum* species.

Based on pairwise similarity data following multiple alignment of all sequences, *G. rostochiensis* and *G. pallida* are as phenetically similar to each other as *G. pallida* is to the Mexican isolates. These data for overall similarity, however, include ancestral similarity as well as phylogenetically useful derived similarity. When simple phenetic clustering algorithms are performed on such data, as is often done with molecular data, the results can be misleading with respect to phylogenetic relationships (16). The PAUP analysis, which uses only phylogenetically informative data, indicated a closer relationship of *G. rostochiensis* to the Mexican isolates.

The results of the present study are consistent with the thesis that Mexico is the center of origin of the potato cyst nematodes (12). This hypothesis suggests that the present-day *Globodera* species evolved from a widespread Laurasian (northern hemisphere) ancestor to account for the centers of diversity in the former Soviet Union and in North America. New evidence suggests that Mexico is also the center of origin of *Phytopthora infestans,* the potato late blight fungus (22). On the basis of molecular and other data, it is now thought that development of the *P. infestans-Solanum* pathosystem probably involved many *Solanum* species in the highlands of central Mexico, which is a secondary center of diversity for the genus *Solanum* and has many endemic *Solanum* species (22). The domesticated *S. tuberosum,* which originated in the Andes Mountains of South America, was not intensively grown in Mexico until the 1950s. Similarly, Mexican *Globodera* species nearly identical to the potato cyst nematodes may have migrated on wild *Solanum* spp. southward to the Andes, with subsequent development on *S. tuberosum,* followed by global transport by man via transport of potatoes.

The many rDNA differences in *G. virginiae,* when compared with the potato cyst nematodes and the two Mexican isolates, suggest that *G. virginiae* also evolved from the postulated (12) widespread Laurasian *Globodera* ancestor, but separately from these Mexican isolates. The three *Globodera* species in the eastern United States may have evolved as a distinct monophyletic group. However, it should be noted that one of us (LIM) observed a similarity in the perineal pattern of the Mexican isolate X-76 and that of *G. solanacearum* (unpubl.). Morphological similarities between *G. virginiae* and a third Mexican *Globodera* isolate, called the Mexican cyst nematode and discussed by Campos-Vela (8), have been reported (24), and we have observed similarities in 2-D protein patterns between *G. virginiae* and a Mexican isolate similar to the Mexican cyst nematode (15). Bossis and Mugniéry (5) noted few morphological differences between the Mexican cyst

TABLE 5. Granek's ratio and length (without neck) to the radial-width ratio of the anterior half of young brown cysts, retained on a 300-μm sieve, of four isolates of *Globodera* species cultured on *Solanum dulcamara.*

Species (isolate)	Granek's ratio	Length/width ratio
G. rostochiensis	3.7 a	1.02 a
X-140	3.2 a	1.03 a
G. pallida	2.6 b	1.10 b
G. virginiae	2.7 b	1.17 b

Data for Granek's ratio are means of $n = 125$ for *G. virginiae,* $n = 100$ for *G. rostochiensis,* and $n = 31$ for X-140 and *G. pallida.* Data for length/width are for means of $n = 51$. Means followed by the same letter are not significantly different ($P = 0.05$) according to Tukey's test.

nematode and *G. virginiae* (as well as *G. tabacum* and *G. solanacearum*), but their detailed quantitative comparisons of 2-D PAGE protein patterns indicated large genetic differences. Their data also indicated that *G. pallida* and the Mexican cyst nematode shared most of their proteins.

The relationship, if any, between similarity in 2-D protein patterns of nematodes and that in rDNA ITS is not clear at present. For the schachtii group of *Heterodera* cyst nematodes, 2-D protein patterns differed markedly, but the rDNA ITS was nearly identical (2,19). In contrast, differences in protein patterns in *H. avenae* strains were paralleled by sequence differences in rDNA ITS (20). This disparity may be an example of mosaic evolution, in which all suites of characters do not evolve in unison in a single taxon or at the same rate in different taxa (16,27). It seems reasonable to assume that a high degree of concordance in postulated relationships, based on a variety of independent data sets, particularly in derived character states, supports those relationships. Detailed phylogenetic analysis based on 2-D protein data is possible (17,21), but is too difficult, time consuming, and costly to be practical in view of the ease with which DNA sequence data can now be obtained and analyzed phylogenetically. Although it is highly probable that protein spots found in all taxa of a study group and in outgroup taxa are ancestral to that group, laborious laboratory methods are necessary to verify the coidentity of a given protein spot and to determine whether it is ancestral or derived (2,5,21).

Additional research with these and other *Globodera* species should further clarify which of the conflicting characteristics among the available data sets, morphological and biochemical, result from conserved ancestral similarities, convergence, or parallel development, and which are phylogenetically informative. The current prevailing view among systematists of most groups of organisms is that the most useful data for establishing phylogenies will be a variety of nucleic acid sequence data from various parts of the genome and organelles (13,25,32). Other kinds of data can then be mapped on a well-corroborated phylogenetic scheme to ascertain patterns of change during evolution.

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