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Phylogenetic Relationships of *Globodera millefolii, G. artemisiae,* and *Cactodera salina* Based on ITS Region of Ribosomal DNA[1]

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Abstract: *Globodera millefolii* and *G. artemisiae* are interesting because their type localities (Estonia and Russia, respectively) are geographically distant from those of the potato cyst nematodes and other *Globodera* species that seem to have originated in the Western world, and because the type host for each is a member of Compositae rather than Solanaceae. Sequence data for ITS1, ITS2, and 5.8S ribosomal DNA (ITS rDNA) for *G. millefolii* and *G. artemisiae* were nearly identical to sequence data for *Cactodera salina* from the rhizosphere of the estuary plant *Salicornia bigelovii* in Sonora, Mexico. The ITS rDNA sequences of these three species were all about 94% similar to those of two other *Cactodera* species for which ITS rDNA data were obtained. Phylogenetic analysis indicated that, based on the ITS rDNA data, *G. millefolii* and *G. artemisiae* are more closely related phylogenetically to the *Cactodera* species than to other nominal *Globodera* species. The molecular data further suggest that the genus *Cactodera* may comprise two or more morphologically similar but separate groups.

Key words: *Cactodera, Cactodera salina, Globodera, Globodera artemisiae, Globodera millefolii,* ITS1, ITS2, nematode, phylogenetic analysis, ribosomal DNA, rDNA, 5.8S gene.

The round-cyst nematode species *Globodera artemisiae* (Eroshenko & Kasachenko, 1972) Behrens and *G. millefolii* (Kirjanova & Krall, 1965) Behrens have been of particular interest to nematologists because their type localities are geographically distant from those of the potato cyst nematodes and other *Globodera* species in the Western world, and also because the type host for each is a member of the Compositae, rather than the Solanaceae. The type locality for *G. artemisiae* is in the Far East Khasan district of Russia, Primorye Territory, and the type host is *Artemisia rubripes* (Eastern Asia wormwood). *Globodera artemisiae* also develops well on *A. vulgaris* (mugwort). The type locality of *G. millefolii* is Talinn, Estonia, and the type host is *Achillea millefolium* (milfoil or common yarrow), although it can develop in the laboratory on a number of Compositae (Krall, 1977). *Globodera artemisiae* does not develop on milfoil. Mature females of both species are circumfenestrate, as is typical for species of both *Globodera* and *Cactodera*. The shape of *G. artemisiae* females is described as egg-like to almost spherical; whereas, the mature female body of *G. millefolii* is described as ovoid (Krall, 1977). A *Cactodera* species (*C. salina* Baldwin, Mundo-Ocampo & McClure, 1997) was found on the estuary plant *Salicornia bigelovii* in Sonora, Mexico. The terminal cone of its females and cysts is so reduced as to approach that of the round cysts of *Globodera* (Baldwin et al., 1997).

Globodera artemisiae has a wide distribution in the Primorye Territory region and also occurs on *Artemisia vulgaris* in northern Germany (Sturhan and Krall, 1991). *Globodera millefolii* is widespread in Estonia and has also been found in Latvia. Krall (1977), after re-examination of the holotype slide, reported errors in the original description and concluded that *G. millefolii* was a distinct species, but that it should be considered a *species inquirenda* until it could be redescribed. The separation of *G. millefolii* from *G. artemisiae* using only morphological data has proven problematical, although the host relationships are different as noted above. Subcultures of both *G. artemisiae* and *G. millefolii* were established on their type hosts in 1970 at Tartu, Estonia, in concrete tubes of more than 1 m diam. *Globodera artemisiae* collected in Nakhodka, Primorye Territory, courtesy of A. S. Eroshenko, has been maintained on

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the type host, *A. rubripes* (which is not found in Estonia and can be maintained only vegetatively in the subculture tubes). *Globodera millefolii,* originally collected on *Tripleurospermum inodora* from Haademeeste, Estonia, has been maintained on its type host, the common milfoil.

The objective of this research was to establish the relationship of *G. artemisiae* and *G. millefolii* to other *Globodera* species. The molecular sequence data for *C. salina* was obtained prior to the initiation of this research at the request of M. A. McClure, and was complementary to an earlier study of rDNA similarities among other *Cactodera* species with which we had worked (Ferris et al., 1995a).

Materials and Methods

Isolates and treatment of samples: The nematode samples containing *G. artemisiae* and *G. millefolii* came from rhizospheres of plants growing in the subcultures established in the experimental garden at Tartu, Estonia, as discussed above. *C. salina* was collected from the rhizosphere of *S. bigelovii* in an experimental plot adjacent to the Centro de Estudios de Desiertos y Oceanos, Puerto Peñasco, Sonora, Mexico. All of these specimens were received in 70% alcohol. We collected *C. weissi* from Pennsylvania smartweed in cultivated fields throughout Indiana and *C. milleri* from lambsquarters in White County, Indiana.

Methods for handling the nematodes and obtaining rDNA were similar to those previously described (Ferris et al., 1993, 1994, 1995b) and are herein summarized. For each sample, a single female or cyst was rinsed in sterile water and crushed in 20 µl cold TE buffer, with a Radnoti (Thomas Scientific, Swedesboro, NJ) 25-µl-size glass homogenizer. The homogenate was either used immediately or stored at −20 °C. From five to 15 such preparations were made for each species. Because of the taxonomic uncertainties surrounding *G. millefolii,* we hand-picked 10 young females of *G. millefolii* from milfoil roots and rhizosphere soil of a single plant, and processed each of the 10 individually. Additional cysts from soil of the experimental garden also were processed.

To prepare homogenate for amplification by polymerase chain reaction (PCR), it was thawed briefly, then microcentrifuged for 3 minutes at 16,000*g*. About 15 µl of the supernatant was discarded, 60 µl Instagene (BioRad) was added to the pellet, and the procedure completed according to the manufacturer's directions. Usually 10 µl of a 1:10 dilution of the preparation was used for each 25 µl PCR reaction, but occasionally this was varied to improve amplification. Standard PCR (Saiki, 1990) was performed with a COY Tempcycler model 50. Primers for PCR amplification were as described previously (Ferris et al., 1993). The amplified region (ca. 1 kb in length) included the two ITS regions (ITS1 and ITS2) plus the 5.8S gene region between them. Multiple amplifications were carried out for each species as discussed above. Amplified rDNA was cloned into the pGEM-T vector (Promega, Madison, WI) and transformed into *E. coli* strain JM 109. Clones were checked for the rDNA insert by PCR, and plasmid preparations were made with the Wizard Plus Miniprep system (Promega). Sequencing was carried out at the Purdue Center for DNA Sequencing using an automatic sequencer (ALFexpress, Pharmacia Biotech). Both strands of multiple clones were sequenced for each species, and the sequences have been deposited in GenBank (AF161003-AF161007).

Sequence analysis: Pairwise comparisons of sequence data for the ITS1, ITS2, and 5.8S areas were made for all species using the computer program GAP in the Sequence Analysis Software Package of the Genetics Computer Group (GCG) (Devereaux et al., 1984) with default penalty values (gap weight = 50, gap length = 3). The sequence data were aligned using the GCG program PILEUP and also with the program Clustal W (Thompson et al., 1994).

Phylogenetic analysis: Phylogenetic analyses were carried out using PAUP*4.0b2a (Swofford, 1998). Taxa included in the analyses were *G. artemisiae, G. millefolii, C. salina, C. weissi* (Steiner) Krall & Krall, and *C. milleri*

Graney & Bird. In addition, the phylogenetic analyses included ITS rDNA from other taxa (*G. pallida* (Stone) Behrens, *G. rostochiensis* (Wollenweber) Behrens and two isolates of the *avenae* group of *Heterodera,* strict *H. avenae* Wollenweber, and a Gotland strain of *H. avenae*) (Ferris et al., 1994, 1995b). The two sequences for *avenae*-group species of *Heterodera* served as the outgroup. With the PILEUP-generated alignment we used maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) algorithms, each of which relies on different assumptions and different ways for solving ties in the data. Although there is no assurance that any of the underlying assumptions are valid (Adams, et al., 1998), it is useful to compare results using different methods of analysis. An analysis with the MP algorithm also was carried out with the data from the alignment from CLUSTAL W. The reliability of trees was tested with a bootstrap test (Felsenstein, 1985) and the Farris JackKnife test, which relies on parsimony (Farris et al., 1995). For the MP analysis, we used the branch-and-bound search, and for the ML analysis we used the heuristic search and the Hasagawa-Kishino-Yano (HKY) model of sequence evolution (Hasagawa et al., 1985). Default parameters were used for one ML test; for a second ML test the parameters used (Ti/Tv = 0.80 and α parameter of gamma distribution = 3.35) were previously estimated from analyses of a similar data set of ITS rDNA. The NJ analyses were performed with the uncorrected "P" distance, the Kimura 2-parameter distance, the Tamura-Nei distance, and the HKY85 distance measures. For the bootstrap analysis of the ML trees we used the heuristic setting with 100 replicates, and for the bootstrap analysis of the MP trees we used the heuristic and the branch-and-bound settings. The branch-and-bound search was used for the JackKnife (parsimony) test. Gaps were treated as missing characters for all analyses. The two *avenae*-group sequences comprised the outgroup taxa in the analyses.

Results

Pairwise nucleotide similarities and dissimilarities among all the species used in the phylogentic analyses are shown in Table 1. The ITS rDNA sequences of *G. millefolii* and *G. artemisiae* were very similar to each other (99.6%), and the rDNA sequence of *G. artemisiae* was identical to that of *C. salina,* except for three nucleotide bases that are present in only one of the two species and missing in the other (Fig. 1). The three *Cactodera* species were less similar (94–95%) to each other. A multiple sequence alignment of the ITS rDNA for all species used in the phylogenetic analyses is shown (Fig. 1). A high degree of nucleotide conservation existed in the 5.8S part of the sequence, with more variability evident in the two ITS areas. *Globodera millefolii* had four base pair differences from *G. artemisiae* that were not shared with *C. salina.*

When the default penalty values for gap

Table 1. Similarity/dissimilarity values in ITS rDNA nucleotide sequence.[a]

	ROS	PAL	ART	SAL	MIL	WEI	CMI	HAV	GOT
ROS	—	3.2	16.6	17.8	18.0	16.1	14.1	30.0	27.2
PAL	96.8	—	15.9	16.2	17.2	15.5	15.3	30.2	27.5
ART	83.4	84.1	—	0[b]	0.4	5.7	6.1	31.6	29.6
SAL	82.2	83.8	100[b]	—	0.4	4.5	6.0	30.8	29.4
MIL	82.0	82.8	99.6	99.6	—	6.3	6.6	30.8	28.4
WEI	83.9	84.5	94.3	94.5	93.7	—	5.6	27.2	26.1
CMI	85.9	84.7	93.9	94.0	93.4	94.4	—	29.0	30.0
HAV	70.0	69.8	68.4	69.2	69.2	72.8	71.0	—	3.2
GOT	72.8	72.5	70.4	70.6	71.6	73.9	70.0	96.8	—

[a] Similarity values are below the diagonal; dissimilarity values are above the diagonal. *Globodera rostochiensis* (ROS), *G. pallida* (PAL), *G. artemisiae* (ART), *Cactodera salina* (SAL), *G. millefolii* (MIL), *C. weissi* (WEI), *C. milleri* (CMI), *Heterodera avenae* (HAV), and Gotland strain of *H. avenae* (GOT).

[b] Gaps treated as missing characters are not considered in calculations of similarity by the program GAP.

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     1                                                            50
ROS  CCCAAGTGAT ACCAATTCAC CACCTACCTG --CTGTCCAG TTGAG-TCAG
PAL  .......... .......... .......... --....T... .....-....
ART  .......... .-.T...... ..GT...... --......C. .....T...A
SAL  .......... ...T...... ..GT...... --......C. .....T...A
MIL  .......... .-.T...... ..GT...... --......C. .....T...A
WEI  .......... ...T...... .......... --......C. .....T...A
CMI  .......... ...T...... .......... --......C. ...T.T.-.A
HAV  .......... T..-...... ..T....... TG........ ....AACGTT
GOT  .......... T..-...... ..T....... TG........ ...GAACGTT

     51                                                           100
ROS  TGTGGGCAAC ACCACATGCC TCCGTTTGTT GTT--GACGG ACACATGCCC
PAL  .......... .......... .......... ...--..... ..........
ART  .......... .......... ........C. .C.--..... G....G....
SAL  .......... .......... ........C. .C.--..... G....G....
MIL  .......... .......... ........C. .C.--..... G....G....
WEI  .......... .......... ........C. .C.--..... .....GA...
CMI  .......... .......... ........C. .C.--..... .....GA...
HAV  GC.T....C. .......... C....C..C. .-------AC G...GGA..G
GOT  GC.T....C. .......... C....C..C. ...GA..... G...GGA..G

     101                                                          150
ROS  GCTGTGTATG GGCTGGCACA TTGACCAACA ATGTACGGAC AGCGC--CCT
PAL  ...A...T.. .......... ....T..... .....T.... .....--...
ART  TAC.-.AGA. .......... .......... C..ATG.... G....CT..C
SAL  TAC.-.AGA. .......... .......... C..ATG.... G....CT..C
MIL  TAC.-.AGA. .......... .......... C..ATG.... G....CT..C
WEI  TAC.-.AGA. .......... .......... C..A.G.... G...TCT..C
CMI  TAC.-.AGA. .......... .......... C..A...... G...GCC..C
HAV  .TC.A.ATG. TCTGT.GG.. CG.GA..... C..AGT.... G..TACC.TG
GOT  .TC.A.ATG. TCTGT.GG.. CG.GA..... C..AG..... G..TACC.TG

     151                                                          200
ROS  GTGCGCATGA GTGTTGGGGT GTAACCGATG TTGGTGGCCC TATGGTGAGC
PAL  .......... .......... .......... .......... A.......CT
ART  ...G...CT. ..-....... .CT..TTGA. .......A.. .....GTG.-
SAL  ...G...CT. ..-....... .CT..TTGA. .......A.. .....GTG.-
MIL  ...G...CT. ..-......C .CT..TTGA. .......G.. .....GTG.-
WEI  ...G...C.. ..-....... .CT..T.-A. .........G ATG.TGA---
CMI  ...G...C.. ..-....... .CT..T..A. ....C..T.. A....GT---
HAV  CGAGCAC.CT ...C.TT..G ..GTT.TCC. AC.A...TG. .TG.TAT.CT
GOT  CGAGCAC.CT ...C.TT..G ..GTT.TCC. .C.A...TG. .TG.TAT.CT

     201                                                          250
ROS  CGACGATTGC TG-------- ---------- ---------- ----------
PAL  .......... ..-------- ---------- ---------- ----------
ART  ----.CCG.. ..-------- ---------- ---------- ----------
SAL  ----.CCG.. ..-------- ---------- ---------- ----------
MIL  ----.CCG.. ..-------- ---------- ---------- ----------
WEI  ----.CCG.T ..-------- ---------- ---------- ----------
CMI  ----.----- A.-------- ---------- ---------- ----------
HAV  GACTCG.... ..AGCAAAGT G-----AAAA GCCTGAGGTT TGGCTGC--G
GOT  GACTCG.... ..AGCAAAGT GATAATAAAA GCCTGAGGTT TGGCTGCGAG
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FIG. 1. Alignment of nucleotide bases of ITS1, 5.8S (italics, positions 601-764), and ITS2 rDNA sequence for *Globodera rostochiensis* (ROS), *G. pallida* (PAL), *G. artemisiae* (ART), *Cactodera salina* (SAL), *G. millefolii* (MIL), *C. weissi* (WEI), *C. milleri* (CMI), *Heterodera avenae* (HAV), and Gotland strain of *H. avenae* (GOT). All base notations are for the nontranscribed strand. Periods indicate sequence identity, and hyphens indicate gaps.

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     251                                                        300
ROS  ---------- ---------- -----CTGTC GTCGGGTCGC TGCACCAACG
PAL  ---------- ---------- -----T.... .......... ..........
ART  ---------- ---------- -----...C. .....C..A. ..T....G..
SAL  ---------- ---------- -----...-. .....C..A. ..T....G..
MIL  ---------- ---------- -----...C. .....C..A. ..T....G..
WEI  ---------- ---------- -----..... .....C.... ..........
CMI  ---------- ---------- -----.C.G. TGTT....A. ..........
HAV  CAAGTCGAGT TGGTGGCGGA CCGCTTGC.G ..T..T.T.. ..T.T.GT..
GOT  GCAATTGAGT TGGTGGCGGA CCGCT.GC.G ..G..T.T.. ..T...GT..

     301                                                        350
ROS  GAGGAAGCAC GCCCACAGGG --CACCCTAA CGGCTATGCT GGCGTCTGTG
PAL  ....TG.... .......... --......... .....G.... ..........
ART  A...TT.... ..T....... GGGCA..C.. .....G.... ..........
SAL  A...TT.... ..T....... GGGCA..C.. .....G.... ..........
MIL  A...TT.... ..T....... GGGCA..C.. .....G.... .C........
WEI  A...T..... ..T....... G---.A.C.. .....G.... ..........
CMI  ....T-.... ..T....... G---.A.C.. .....G.... ..........
HAV  TT...C.AG. A.G.TGTT.. G-T.A..C.. .....G.... ..T.......
GOT  A...G..... ..T.-GTT.. G-T.A..C.. .....G.... ..T.......

     351                                                        400
ROS  C--CGTTGAG CGGTTGTTGC GCCTCGC--- AGATATGCTA ACATGGAGTG
PAL  .GT....... .......... CTTG...--- G........G .......A..
ART  .GT....... T......... ....T..GTG G..C..A..G .TG..T.AGT
SAL  .GT....... T......... ....T..GT- G..C..A..G .TG..T.AGT
MIL  .GT....... T......... ....T..GTG G..C..A..G .TG..T.AGT
WEI  .GT....... .......... ....T..--T G..C..A..G ..G....AGT
CMI  .GT....... .......... ....T..--T G..C..A..G ..G....AGA
HAV  .GT....... .........T ....G..ACA T.TG.CA.-. CTGGCCT.G.
GOT  .GT....... .........T ....G..ACA T.TG.CA.-. CTGGCCT.G.

     401                                                        450
ROS  TA-GCTG-CT ACTCCATGTT GTACGTGCCG TACCTTGCGG CATGTCTGCG
PAL  ..G....-.. .T........ .......... ....-C.... ..........
ART  ---.T..TAC CT.......C ..G.CAT.G. -...CA.... T.......T.
SAL  ---.T..TAC CT.......C ..G.CAT.G. -...CA.... T.......T.
MIL  ---.T..TAC CT.......C ..G.CAT.G. -...CA.... T.......T.
WEI  CT-.T..TAC CT....C..C ....C.A.T. ....CA.... T.......T.
CMI  GTG.TC.TAC CT...TC..C ....CA.TG. ....CA.... T.......T.
HAV  A.A.TG.TTC TT...TG.CC T....AA... ..A..A.... TG......T.
GOT  A.G.TG.TTC TT...TG.CC T....AA... ..A..A.... TG......T.

     451                                                        500
ROS  CTTGTGTGCT ACGTCCGTGG CCGTGATGAG ACGACGTGTT AGGACCCGTG
PAL  .......... .........A .......... .......... ..........
ART  .......... .......... .......... .......... ..........
SAL  .......... .......... .......... .......... ..........
MIL  .......... .......... .......... .......... ..........
WEI  .......... .......... .......... .......... .......A..
CMI  .......... .........A .......... .......... ..........
HAV  .C........ .......... .......... ........G. ...G......
GOT  .......... .......... .......... ........G. ...GT.....
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FIG. 1. *(Continued).*

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     501                                                        550
ROS  CCTTGGCATT GGCACGTGGT TTAAGACTTG ATGAGTGCCC GCAG--CAC-
PAL  ..-....... .......... .......... .......... ....--...-
ART  ...G.CT-.. ....T..... .........CA .........T. .GT.TG...C
SAL  ...G.CT-.. ....T..... .........CA .........T. .GT.TG...C
MIL  ...G.TT-.. ....T..... .........CA .........T. .GT.TG...C
WEI  ...G.CT..G ....T..... .........CA .........-. AG.CAG...C
CMI  ...G.C-... ....T..... .........CA .........-. CG.CAG...C
HAV  .TA..CTTC. -.........C .........CA .......T.. ..TAGG...C
GOT  .TC..CTTA. A.........C ..........A .......T.A ..TAGG...C

     551                                                        600
ROS  GCCAGCTTTT TCCCATTTTT ATTTATTTTT T-ATGCAATT CGATTGCTAA
PAL  .......... ..T....... ..AA...... .A........ T.........
ART  .....TG... ..T.......C .A.A.-.... .TTATGC.AC ATTG.TGCT.
SAL  .....TG... ..T.......C .A.A.-.... .TTATGC.AC ATTG.TGCT.
MIL  .....TG... ..T.......C .A.A.-.... .TTATGC.AC ATTG.TGCT.
WEI  ......G... ..T.......C .A.A.-.... .TTGTGC.AC TCTG.TGCT.
CMI  .....TG... ..T.......C .A.A...... .TTACGC.AC ATTG.TGCT.
HAV  .....TG... .TTTTCA.... ....T...GA CC.CTTCT.. GTTGAAGA..
GOT  .....TG... .TTTTCA.... ....T...GA CC.CTTCT.. GTTGAAGG..

     601                                                        650
ROS  AATATTCTAG TCTTATCGGT GGATCACTCG GCTCGTGGAT CGATGAAGAA
PAL  .......... .......... .......... .......... ..........
ART  .......... .......... .......... .......... ..........
SAL  .......... .......... .......... .......... ..........
MIL  .......... .......... .......... .......... ..........
WEI  .......... .......... .......... .......... ..........
CMI  .......... .......... .......... .......... ..........
HAV  G.A....... .......... .......... .......... ..........
GOT  G.A....... .......... .......... .......... ..........

     651                                                        700
ROS  CGCAGCCAAC TGCGATAATT AGTGTGAACT GCAGAAACCT TGAACACAGA
PAL  .......... .......... .......... .......... ..........
ART  .......... .......... .......... ........T. ..........
SAL  .......... .......... .......... ........T. ..........
MIL  .......... .......... .......... ........T. ..........
WEI  .......... .......... .......... ........T. ..........
CMI  .......... .......C.. .......... ........T. ..........
HAV  .......... .......... ....C..... .......... ........A.
GOT  .......... .......... ....C..... .......... ........A.

     701                                                        750
ROS  ACTTTCGAAT GCACATTGCG CCATTGGAGT GACATCCATT GGCACGCCTG
PAL  .......... .......... .......... .......... ..........
ART  ..C....... .......... .......... A.T....T.. ..........
SAL  ..C....... .......... .......... A.T....T.. ..........
MIL  ..C....... .......... .......... A.T....T.. ..........
WEI  .......... .......... .......... A.T....T.. ..........
CMI  .......... .......... .......... A.T....T.. -.........
HAV  .......... .......... .......... T..........  ..........
GOT  .......... .......... .......... T.......... ..........
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FIG. 1. *(Continued).*

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     751                                                                800
ROS  GTTCAGGGTC GTAACCAAAA AATGCACTGC ATGTGCGTGT TTTATTTGCT
PAL  .......... .......... .......... .......... ..........
ART  .......... .......... .........A .......... ...GAC....
SAL  .......... .......... .........A .......... ...GAC....
MIL  .......... .......... .........A .......... ...GAC....
WEI  .......... .......... .......... .......... ....AC....
CMI  .......... .........T .......... .......... ...GAC....
HAV  .......... ..T.T..T.. ..GCACTGCT G..CCT..TA .G.TGG..AG
GOT  .......... ..T.T..T.. ..GCACTGCT G..CCT..TA .G.TGG..AG

     801                                                                850
ROS  AAGAT-ACGC TTCGGCGTGT TCTTGCATAC TATTGAATCG TACGCTGTGT
PAL  .....-.... .......... .......... C......... ..........
ART  .....C.... CCG.T..... .........A AGC.-.-.G. ..........
SAL  .....C.... CCG.T..... .........A AGC.-.-.G. ..........
MIL  .....C.... CCG.T..... .........A AGC.-.TGGC ..........
WEI  G....C.... CCG.T..... .........A AGC.-.TG.. ..........
CMI  .....C.... C.G.T..... .........A A.C.-.TG.. ..........
HAV  .TC..GT..G CAT.A..... ......GCTA .TC.TG.AAA .G.T.G.CCG
GOT  .TC..GT.TG CAG.A..... ......GCTA .TC.TG.AAA .G.T.G.CCG

     851                                                                900
ROS  AGCGTTGGAC GTCGTGGCGC GAAAATGTGT TG---TCATT CGCGCTTTAC
PAL  ..T..... .......... .......... ..---..T.. ..........
ART  .......... ..GC...T.. .G........ ..TTT..T.C ..T.......
SAL  .......... ..GC...T.. .G........ ..TTT..T.C ..T.......
MIL  .......... ..GC...T.. -G........ ..-TT..T.C ..T.......
WEI  .. ....... ..GC...T.. .G........ ..TTC-.T.C ..T.......
CMI  G......... ..GC...T.. .G........ ..TTC..T.C ..T.......
HAV  T.GAG..TGG T.GTGTTG.. .CG..AC... CAGGT..T.. ....T.....
GOT  T.GAG..TGG T.GTGTTG.. .CG..AC... CAGGT..T.. ....T.....

     901                                                                950
ROS  AGACCGTAAT TTAGGCACGC CCTTCGTTCA CATGCGATAG CTGAATGCCT
PAL  .......... .......... .......... .......... ..........
ART  .......... ......G..T ..C.T..... .G..T..... ..A.......
SAL  .......... ......G..T ..C.T..... .G..T..... ..A.......
MIL  .......... ......G..T ..C.T..... .G..T..... ..A.......
WEI  .......... ......G..T ..A....... T......... ..A.......
CMI  .......... .....T...T ..C....... T......... ..A.......
HAV  G.T......C .AG.A.G.AA .TGCTCGC.. TG..T.C.G. G..G.ACTG.
GOT  G.T......C .AG.A.G.AA .TGCTC.C.. TG..T.C.G. G..G.ACTG.

     951                        977
ROS  CGCCAATAGG CATTTGCAAT TGAACAT
PAL  .......... .......... .......
ART  .......... ......T.C. ...CTGC
SAL  .......... ......T.C. ...CTGC
MIL  .......... ......T.C. ...CTGC
WEI  G.G.G..... ......T.C. ...CTGC
CMI  .......... ......T.C. ...CTGC
HAV  ...TGG.... ....CCGTG. .TG.AT.
GOT  ...TGG.... ....CCGTG. .TG.AT.
```

FIG. 1. *(Continued).*

weight (5.0) and gap length (0.3) were used in PILEUP, visual inspection revealed no problem areas in the data and no rationale for using different penalty values. Whatever the order of data entry, PILEUP reorders the data into a new order that is based on overall similarity in the data and produces a dendrogram based on clustering relationships of the aligned sequences. Although it is possible to override this reordering of the data, the dendrogram of phenetic relationships appeared also to be a good initial assessment of probable phylogenetic relationships among the species and species groups. This approach for generating an alignment results in a phylogenetic weighting to maintain what we considered to be the genealogical basis for homology assessment (Mindell, 1991). The alignment with the program CLUSTAL W (with default values) was generated with random entry of taxa.

The best tree from both ML analyses, and the trees from the NJ analyses (with each of the four distance measures used), had the same topologies (Fig. 2). All of these trees showed a trichotomy for the clade containing *G. artemisiae, G. millefolii,* and *C. salina,* as might be expected from the similarity in their ITS rDNA data (Table 1). A total of six "minimum length" trees of 527 steps were obtained with the MP analysis, each of which differed slightly in the relationships shown for the three *Cactodera* species and the two nominal *Globodera* species. Three of these MP trees had a *C. weissi* + *C. milleri* clade (as in Fig. 2), and three other trees showed *C. weissi* and *C. milleri* separately joining a clade comprised of *G. artemisiae, G. millefolii,* and *C. salina,* with varied branching patterns among the three MP trees. The MP bootstrap analysis with heuristic settings showed the topology of Figure 2, with a bootstrap value of 52% for the branch leading to the *C. weissi* + *C. milleri* clade. The MP bootstrap analysis with branch-and-bound settings and the Farris JackKnife analysis, however, resulted in unresolved separate branches for *C. weissi* and *C. milleri* that joined a trichotomy for the clade containing *G. artemisiae, G. millefolii,* and *C. salina* (Fig. 3). The topology of the bootstrap analysis (branch-and-bound) of the MP tree generated from the CLUSTAL W alignment was the same as in Figure 2.

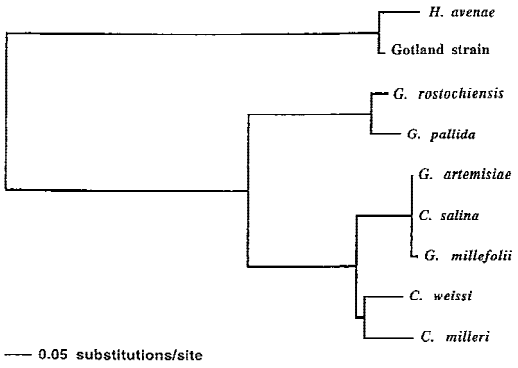

FIG. 2. Best maximum likelihood (ML) tree (–Ln likelihood = –3595.95583) for *Cactodera, Globodera,* and *Heterodera* spp. based on ITS rDNA of sequences of Figure 1 with parameters set at Ti/Tv = 0.80 and α-parameter of gamma distribution = 3.35. Branch lengths are proportional to the number of inferred changes. Gotland strain is putative *H. avenae.*

DISCUSSION

The extreme ITS rDNA similarity among *C. salina, G. millefolii,* and *G. artemisiae* was not expected. Inasmuch as these molecular data sequences are nearly identical for the three species, their relationships to each other cannot be be resolved with confidence by phylogenetic analyses based solely on the ITS rDNA data. In all of the analyses, however, they were linked more closely to the other two *Cactodera* species than to the clade comprised of *G. pallida* + *G. rostochiensis.*

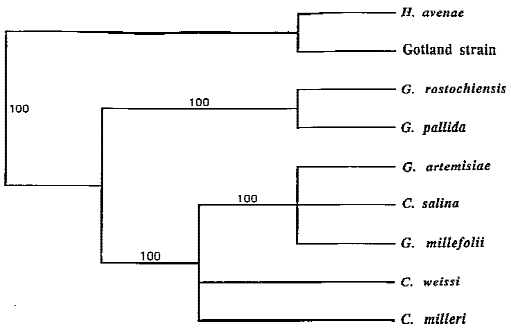

FIG. 3. Consensus tree from the maximum parsimony (MP) bootstrap analysis for *Cactodera, Globodera,* and *Heterodera* spp. Bootstrap figures are listed on branches. Gotland strain is putative *H. avenae.*

It is likely that the ITS rDNA data reflect species relationships and, if so, it appears that *G. millefollii* and *G. artemisiae* are more closely related phylogenetically to *Cactodera* species than to other nominal *Globodera* species. Despite the nearly identical ITS rDNA, *C. salina* is undoubtedly a species distinct from the Estonian and Russian *Globodera* spp., based on geographic location as well as ecological and host differences. Ferris et al. (1993) have shown that distinct congener species may be very similar in their ITS rDNA, which appears to be highly conserved in cyst nematodes. Although *G. millefolii* and *G. artemisiae* are found in closer geographic proximity to each other, their host differences, plus the four base pair differences (in addition to some missing bases in one or the other species), suggest that they, too, are distinct species. Further information based on less-conserved DNA sequence data will help to test these conclusions. As more DNA sequence data become available for *Cactodera* species, the genus may need to be subdivided into two or more closely related groups that share similar morphological characteristics but differ sufficiently in molecular and other characteristics (e.g., host and ecological differences) to make such division reasonable and desirable. New molecular data will provide additional guidance for appropriate taxonomic changes, which should be based on a wider spectrum of molecular data than we have at present, as well as on all other known data about the species. Additional molecular data will probably be more useful in determining phylogenetic relationships among these species than will further manipulations of the molecular data currently available.

The topic of co-evolution of plants and their nematode parasites (Krall and Krall, 1970, 1978, 1983) continues to interest nematologists. Although corroborated phylogenies essential for the study of co-evolution have not yet been developed for most plant groups, many of the major plant groupings recognized by classical botanists (e.g., Dahlgren, 1980) are being corroborated by new molecular data (Chase et al., 1993; Rice et al., 1997; Soltis et al., 1997, 1998). The classic and current data taken together suggest that the host plants for the cyst nematode groups in this study are taxonomically well separated. *Cactodera salina* and *C. milleri* have hosts in the family Caryophyllidae, a plant family that is well supported by classical data and many molecular data (Dahlgren, 1980; Soltis et al., 1997, 1998). Classic and current molecular analyses also agree that the *Polygonum* host of *C. weissi* is near the Caryophyllidae. Hosts for *G. millefolii* and *G. artemisiae* are in one group of asterids (sensu stricto), whereas the solanaceous hosts of other nominal *Globodera* species are placed in another group, the lamiids. As more phylogenies are developed for plant and nematode groups, study of co-evolution of hosts and parasites may become more tractable.

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