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Research Note

Characterization of Two *Steinernema scapterisci* Populations (Nemata: Steinernematidae) Using Morphology and Random Amplified Polymorphic DNA Markers

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ABSTRACT: The entomopathogenic nematode, *Steinernema scapterisci* (Rhabditida: Steinernematidae), was originally isolated from the mole cricket *Scapteriscus vicinus* (Orthoptera: Gryllotalpidae) in Uruguay. Subsequently, a population of *S. scapterisci* was isolated from the mole cricket *S. borellii* in Colon, Buenos Aires, Argentina. Because of the distance between the nematode isolates from Uruguay and Argentina and the different *Scapteriscus* species from which they were isolated, a study to examine the possible heterogeneity of *S. scapterisci* populations over space was conducted. Morphological variation was correlated with random amplified polymorphic DNA markers.

KEY WORDS: *Steinernema scapterisci*, Argentina, Uruguay, morphometrics, RAPD's, genetic variation, principal component analysis.

The entomopathogenic nematode, *Steinernema scapterisci* Nguyen and Smart, 1990 (Rhabditida: Steinernematidae), shows potential for biological control of mole crickets in the genus *Scapteriscus* Scudder in the southeastern United States (Parkman and Frank, 1992; Parkman et al., 1993, 1994). Mole crickets, accidentally introduced into North America in the early 1900's from South America (Walker and Nickle, 1981), cause extensive damage to turfgrass. *Steinernema scapterisci* initially isolated from Uruguay from *Scapteriscus vicinus* Scudder (Nguyen and Smart, 1990) was subsequently released in Florida to control mole crickets. It has become established but does not control the cricket populations (Parkman and Frank, 1992).

Stock (1992) isolated *S. scapterisci* from *Scapteriscus borellii* Giglio-Tos in Colon and Pergamino, Argentina, located in the Province of Buenos Aires approximately 500 km from the Uruguayan border. This isolate was propagated by industry (biosys, Palo Alto, California) and designated as Argentinian strain 319. We obtained the Uruguayan strain from Dr. Grover Smart, University of Florida, Gainesville, Florida. This Uruguayan isolate had been designated previously by biosys as strain 292. Because of the geographic distance between the 2 nematode isolates from Uruguay and Argentina, and because they were isolated from different *Scapteriscus* species, we conducted experiments to determine whether or not there were morphometric and DNA differences between the 2 populations.

The methods for rearing both nematode isolates were similar. We used standard in vivo culture techniques with the house cricket *Acheta domesticus* L. (Orthoptera: Gryllidae) as the host organism. First- and second-generation adults were obtained by dissecting infected house crickets 3–4 and 6–8 days, respectively, after they died. Infective juveniles were recovered when they emerged from the cadavers in a modified White trap (Woodring and Kaya, 1988), in 8–14 days. For morphometrics, nematodes were fixed in TAF and cleared in lactophenol (Gardner et al., 1994).

Quantitative measurements were made using a Leitz Ortholux II microscope with an ocular micrometer and Jandel® software or video imaging system. Standard descriptive statistics and principal component analysis (PCA) were used for analysis (SAS Institute, 1988).

Random amplified polymorphic DNA (RAPD) fragment analysis was performed to assess the extent of interpopulation genetic variation following the method of Caswell-Chen et al. (1992) and Gardner et al. (1994) with the following modifications: several thousand infective juveniles from each population collected from the modified White trap were separately washed in buffered saline (9%) 3 times. Centrifugation flotation, using 30% sugar solution, was used to further clean the nematodes, followed by 3 washes in sterile water. After washing, the infective juveniles were frozen quickly in liquid nitrogen and

Table 1. Comparison on the biometrics of males of Argentinian and Uruguayan populations of *Steinernema scapterisci*.

	Argentinian strain (n = 20)											
	Present study						Stock (1992)					
	First Generation			Second Generation			First generation			Second generation		
Character*	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	1,597	131	1,355–1,800	993	153	850–1,423	1,500	250	1,000–1,900	1,000	80	989–1,300
Width (W)	133	16	108–166	73	18	55–122	130	42	90–200	65	9	57–76
Stoma L	5	0.6	4–6	4	0.5	3–5	4	0.3	4–5	3.8	1	3.5–5.5
Stoma W	6	0.7	5.5–7.5	5	0.5	4–6	5.7	1	4.5–7	5	1.2	4.5–6.5
AE-EP	85	9	67–109	64	7	53–77	65	11	60–89	63	8	54–78
AE-NR	130	14	109–172	118	12	99-143	128	10	120–140	110	10	96–129
AE-P	181	11.5	164–203	167	12	142–184	175	12	150–198	152	13	135–185
Tail L	30	3	23–35	24	3.5	17–31	23	5	19–27	23	3	19–26
Cloaca W	42	4	33–50	28	4	21–39	29	4	26–39	27	4	20–35
Mucron L	3	0.5	2–4	3	0.4	2–4	3.8	0.5	2.8–4.5	3	0.6	2.8–3.7
Testis refl.	326	30	258–368	157	20	102–197	356	39	298–395	189	18	160–215
Spicule L	91	4	83–99	74	6	63–87	80	5	72–89	77	4	74–83
Gub. L	59	7	50–75	53	6	43–67	59	4	57–70	50	4	45–59
	Uruguayan strain (n = 20)											
	Present study						Nguyen and Smart (1990)					
	First Generation			Second Generation			First generation			Second generation		
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	1,745	355	1,337–2,281	1,150	90	1,084–1,354	1,728	358	1,319–2,271	1,174	95	1,031–1,342
Width (W)	163	45	99–235	74	10	65–87	156	49	97–231	73	8	62–84
Stoma L	4.5	1	3–5	4.5	1	3–5.5	4.4	1	3–5	4.3	1	3–6
Stoma W	6.6	1.5	5.5–8	6	1	5–7.5	6.1	1	5–8	6	1.2	5–8
AE-EP	75	12	66–96	66	6	54–74	71	11	63–98	68	7	50–75
AE-NR	138	10	125–149	120	9	100–129	136	11	120–152	121	10	103–131
AE-P	190	18	162–212	170	11	140–178	187	21	164–216	168	13	138–181
Testis refl.	375	48	311–445	200	17	180–235	374	52	306–447	205	19	176–234
Tail L	22	3	20–28	23	3	20–28	25	3	21–30	25	3	22–30
Mucron L	4	0.5	2.9–4.5	3.9	0.4	3.0–4.4	4.3	0.6	3.1–4.7	3.9	0.6	3.1–4.6
Cloaca W	32	4	30–42	31	3	27–40	33	5	31–45	33	4	28–41
Spicule L	84	4	72–90	79	4	71–81	83	5	72–92	78	3	75–83
Gub. L	63	4	58–74	53	3	49–57	65	5	59–75	54	3	47–59

* Abbreviations: AE-EP = distance from tip of head to excretory pore; AE-NR = distance from head to nerve ring, AE-P = distance from head to pharynx base.

Table 2. Comparison on the biometrics of infective juveniles of Argentinian and Uruguayan populations of *Steinernema scapterisci*.

	Argentinian strain (n = 20)					
	Present study			Stock (1992)		
Character*	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	524	29	467–568	530	29	500–570
Width (W)	27	2	22.5–31.5	20	3	15–25
AE-NR	78	5	69–86	89	1.1	80–97
AE-EP	38	2	34–42	36	4	34–42
AE-P	118	8	105–136	120	4	114–142
RD	0.32	0.03	0.25–0.34	0.4	0.03	0.30–0.46
RE	0.76	0.06	0.75–0.78	0.7	0.05	0.63–0.75
Tail L	48	2	45–53	49	4	47–54
	Uruguayan strain (n = 20)					
	Present study			Nguyen and Smart (1990)		
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	580	27	517–615	572	27	517–609
Width (W)	32	9	17–31	24	4	18–30
AE-NR	95	9	79–112	97	1.1	83–106
AE-EP	43	5	36–50	39	4	36–48
AE-P	125	7	111–136	127	6	113–134
RD	0.34	0.04	0.28–0.41	0.31	0.03	0.27–0.40
RE	0.8	0.09	0.64–0.98	0.73	0.06	0.60–0.80
Tail L	54	5	44–62	54	3	48–60

* Abbreviations: AE-NR = distance from tip of head to nerve ring; AE-EP = distance from head to excretory pore, AE-P = distance from head to pharynx base, RD = AE-EP/AE-P, RE = AE-EP/tail length.

stored at −80°C until processed for DNA analysis.

The frozen nematodes were transferred to a glass tissue homogenizing tube containing extraction buffer (1% sodium lauryl sulfate; 50 mM; ethylenediaminetetraacetic acid (EDTA); 100 mM Tris-HCl, pH 8; 200 mM NaCl; 50 μg/ml proteinase K), homogenized on ice at 1–2°C, and transferred to a 1.5-ml Eppendorf® tube. Extraction buffer was added to make a final volume of 300 μl. This was incubated in a water bath at 55°C for 2 hr. To remove proteins and other cellular debris, equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) were added to the tube and centrifuged at 16,000 *g* for 15 min at room temperature. The extraction procedure was repeated again, and the DNA was precipitated from the supernatant portion with 2.5 volumes of cold 95% ethanol. The precipitate was resuspended in polymerase chain reaction in (PCR) TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) calibrated at 10 μg/μl and used as the DNA template for amplification using the PCR for the RAPD analysis.

Operon® primers (A-05 and A-11) 10 nucleotides in length were used for all reaction experiments with an annealing temperature of 35°C. Purified DNA from the nematode genome was subjected to the PCR, and the amplified DNA was electrophoresed on a 1.7% horizontal agarose gel. PCR products were photographed after staining with 2 μl/ml ethidium bromide for 10 min.

The isolates and/or species included on the gel were the following: *S. carpocapsae* Weiser (All strain), *S. glaseri* Steiner, *S. scapterisci* (Argentinian isolate 319 and Uruguayan isolate 292), *Heterorhabditis hawaiiensis* Gardner, Stock and Kaya, 1994, and *H. indicus* Poinar, Karunakar, and David, 1993.

Amplification products were checked for DNA contamination from the nematodes bacterial symbiont (Caswell-Chen et al., 1992), and none of the nematodes' RAPD patterns included the bacteria's DNA. Throughout this study, RAPD reactions were always duplicated and care was taken to ensure consistency in DNA banding profiles between replicates and between separate experimental runs.

PCA was performed on morphometric vari-

Table 3. Comparison on the biometrics of females of Argentinian and Uruguayan populations of *Steinernema scapterisci*.

	Argentinian strain (n = 20)											
	Present study						Stock (1992)					
	First Generation			Second Generation			First generation			Second generation		
Character*	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	4,740	1,367	3,997–6,534	2,171	222	1,775–2,500	3,890	450	3,020–3,970	2,015	224	1,786–2,347
Width (W)	198	57	169–276	134	19	100–167	164	12	153–190	112	16	86–135
Stoma L	9	2	8–12	5	1	4–6	6.8	1	5–8.5	6	1.5	5–8
Stoma W	12	3	10–14	6.5	0.5	6–7.5	8.5	3	7–12	7.5	0.8	7–10
AE-EP	99	3	88–149	76	6	62–85	78	5	75–90	70	7	63–84
AE-NR	175	44	148–231	157	15	154–259	153	11	144–170	146	11	139–168
AE-P	260	65.5	230–366	206	24	198–242	215	14	198–240	210	12	196–230
V%	51	12	50–60	55	2	50–58	53	2	49–54	55	2	53–57
Tail L	63	15	55–78	54	3	47–59	42	5	30–47	47	3	39–58
Anus W	67	18	60–96	52	8	33–65	52	3	40–62	41	5	39–51
	Uruguayan strain (n = 20)											
	Present study						Nguyen and Smart (1990)					
	First Generation			Second Generation			First generation			Second generation		
	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Range
Length (L)	4,247	574	3,544–5,219	2,220	231	1,996–2,545	4,162	540	3,531–5,156	2,209	223	1,841–2,530
Width (W)	184	12	161–210	150	17	100–165	179	13	159–203	123	14	94–141
Stoma L	7	1	6–9	7	1.5	6–9	7.5	1	6–9	6.7	1.4	5-9
Stoma W	10	2.5	9.5-12	9	1.1	8.5–12	10	3	9–12	8.9	0.9	8–11
AE-EP	92	4	80–98	80	7	65–90	89	5	78–94	78	6.8	66–88
AE-NR	179	11	158–195	172	12	150–187	174	13	153–194	169	12	147–184
AE-P	240	16	220–273	245	13	230–267	242	17	219–269	241	15	222–266
V%	53	2	51–54	53	2	52–59	53	2	50–54	52	2	52–60
Tail L	44	9	38–58	57	3	47–62	46	8	34–59	58	4	48–64
Anus W	56	7	46–70	45	3	50–59	58	9	41–72	47	2.8	43–52

* Abbreviations: AE-EP = distance from tip of head to excretory pore; AE-NR = distance from head to nerve ring, AE-P = distance from head to pharynx base.

Table 4. PCA eigenvectors.

Variables*	Males first generation			Males second generation		
	PC I	PC II	PC III	PC I	PC II	PC III
LLENGTH	0.295839	0.351143	0.055837	**0.458753**	0.104773	−0.257540
LWIDTH	0.295271	0.321798	0.031507	0.312456	0.154789	0.057423
LSTL	−0.134271	0.213577	0.541455	−0.095463	−0.09874	0.321456
LSTW	−0.009748	0.344268	**0.588545**	−0.014537	0.214568	0.231114
LAEEP	−0.040713	**0.451225**	−0.466217	0.317662	0.222009	−0.546420
LAENR	0.191067	0.266521	−0.311030	0.038308	0.449822	0.089736
LAEPH	0.242651	0.407037	0.022556	0.304647	0.052375	**0.510891**
LTAILL	**0.376394**	−0.134926	0.000515	0.127584	0.477967	−0.160825
LMUCL	**0.374418**	−0.120605	0.015726	−0.007926	**0.595884**	0.420361
LWANUS	−0.375566	0.160077	−0.043911	0.411356	−0.188966	0.386077
LTREF	−0.374313	0.141142	0.014545	0.284663	−0.244254	−0.024976
LSPICL	−0.283503	0.300227	−0.186097	0.388296	0.082999	−0.080399
LGUBL	0.258314	−0.016155	0.068581	0.420412	−0.221052	0.08559
	Females first generation			Females second generation		
	PC I	PC II	PC III	PC I	PC II	PC III
LLENGTH	0.316118	−0.256909	0.338031	0.268249	0.437956	−0.249719
LWIDTH	0.32629	−0.350363	−0.152237	0.237282	0.423147	−0.077213
LSTL	0.350094	0.183427	−0.277429	0.419417	−0.299813	0.151857
LSTW	0.299056	0.390519	−0.303922	0.447163	−0.241607	0.252938
LAEEP	0.328718	−0.097695	0.246767	0.195385	**0.517416**	−0.084405
LAENR	0.269963	−0.478798	0.259357	0.421995	0.035068	0.012652
LAEPH	**0.371723**	−0.055152	−0.053824	**0.461802**	−0.149729	−0.028579
LTAILL	0.361324	0.294745	−0.119113	0.013954	0.406327	0.492631
LWANUS	0.348978	0.103314	−0.089645	0.237042	−0.105532	0.012395
LVUL	0.100745	**0.534519**	**0.734808**	−0.099867	0.115204	**0.770519**
	Infective juveniles					
	PC I	PC II	PC III			
LLENGTH	**0.457758**	−0.054397	−0.099386			
LWIDTH	−0.065448	−0.272419	**0.923116**			
LAEEP	**0.416861**	−0.105271	−0.117848			
LAENR	0.441831	0.301795	0.168456			
LAEPH	0.374406	−0.277930	−0.061180			
LRA	0.243034	**0.503654**	0.277628			
LRB	0.045611	**0.633476**	0.083024			
LTAILL	0.419901	−0.297427	0.089444			

* See text for definition of acronyms. Boldface indicates dominant eigenvector.

ables representing mensural data of the pooled males and females of first- and second-generation and infective juveniles from the Argentinian and Uruguayan populations (Tables 1–3). Eigenvectors of all the characters of the infective juveniles, male and female first generations and male and female second generations contributing to the 3 principal components (PC I, PC II, PC III) are presented in Table 4.

Within the first-generation males, variables have relatively small values in PC I; the negative values indicate negative covariation of those characters with the other character values. PC II is influenced most by the distance from head to excretory pore (LAEEP) and the distance from head to pharynx base (LAEPH), whereas PC III is mainly influenced by the stoma width (STW). PC I of second-generation males is influenced by the total length (LLENGTH), whereas PC II and III are most influenced by the length of the tail (LTAILL) and the distance from head to nerve ring (LAENR), respectively.

Eigenvectors of the variables of first- and second-generation females show that PC I and PC III are dominated by the distance from head to pharynx base (LAEPH) and V% (LVUL), respectively, whereas PC II is influenced by V% (LVUL) in first-generation females and the dis-

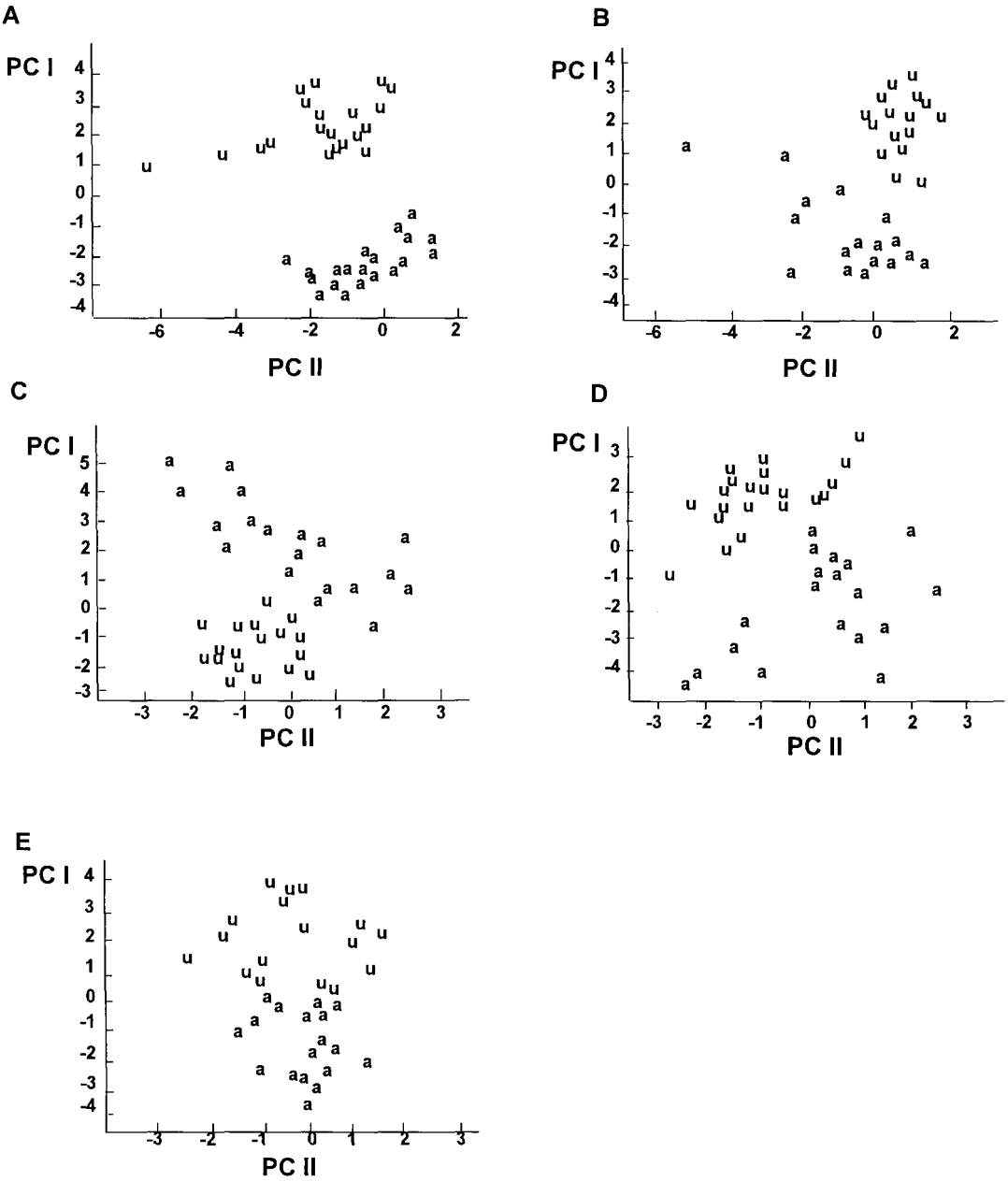

Figure 1. Scatter plots of PCA showing the clustering of the Argentinian (a) and Uruguayan (u) populations of *Steinernema scapterisci* by means of PC I and PC II of the matrix of the morphometric characters of each nematode stage. A. Males first generation. B. Males second generation. C. Females first generation. D. Females second generation. E. Infective juveniles.

tance from head to excretory pore (LAEEP) in second-generation females.

Within the infective juveniles, all variables have positive values except the width (LWIDTH), which indicates that this character has a negative covariance with the rest of the variables in the data set. It appears to show that PC II is dominated by ratio A (LRA) and ratio B (LRB) and PC III is mostly influenced by width (LWIDTH).

Results generated by the statistical analysis us-

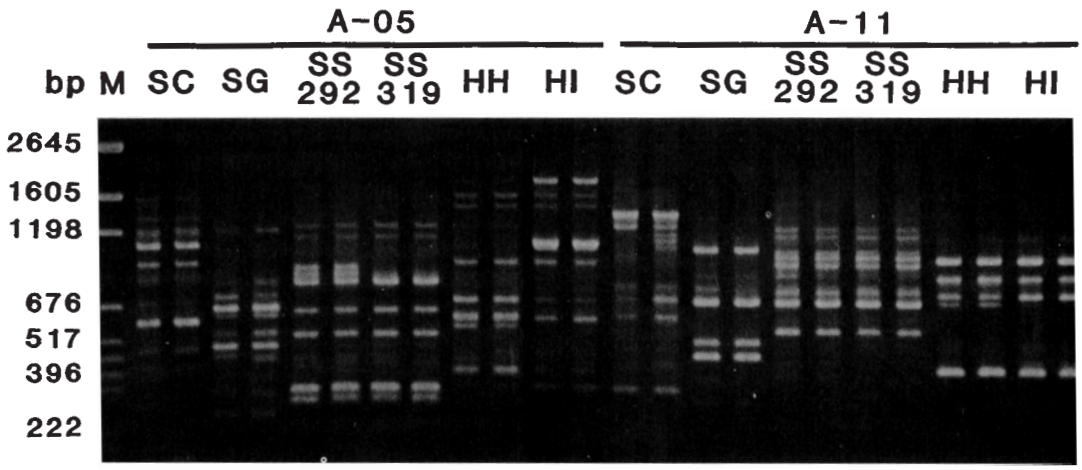

Figure 2. RAPD fragments from isolates of 4 species/isolate of *Steinernema* and 2 species of *Heterorhabditis*. For each presumptive species/isolate, the sample was duplicated on the gel to check consistency; thus, there are 2 lanes on the gel for each species/strain, except for the molecular size standard in the first lane, M. From left to right: bp = base pairs; operon primer A-05: M = lane 1, the molecular size RAPD standard; SC = lanes 2 and 3, *Steinernema carpocapsae*; SG = lanes 4 and 5, *S. glaseri*; SS 292 = lanes 6 and 7, *S. scapterisci* from Uruguay; SS 319 = lanes 8 and 9, *S. scapterisci* from Argentina; HH = lanes 10 and 11, *Heterorhabditis hawaiiensis*; HI = lanes 12 and 13, *H. indicus*; operon primer A-11; lanes 14–25, same sample order as in operon primer A-05.

ing PCA show that there are significant quantitative morphological differences between the Uruguayan and Argentinian populations, which are illustrated by scatter plots of PC I vs. PC II (Fig. 1A–E). It is evident that, given the variables used in the analysis, PCA provided good separation of the individuals of these 2 populations.

Analysis of the RAPDs (using operon primer A-05) showed that there were some differences in the band patterns between the Argentinian and Uruguayan populations of *S. scapterisci*.

The differences observed were between the range of 676 and 1,198 base pairs of the molecular size standard marker (Fig. 2). No differences could be demonstrated using operon primer A-11.

Even though a minor variation in the band patterns was generated by 1 of the markers when comparing the 2 populations, the analysis of genetic variation using RAPDs is well suited for use in population genetics and studies of biodiversity (Waugh and Powell, 1992).

This study shows that there is significant heterogeneity in *S. scapterisci* populations in space.

Careful examination of these nematodes should reveal further heterohomogeneity in the morphological and genetic characteristics in different populations. Thus, in our study, the combination of molecular techniques and classical morphological studies was a useful tool to evaluate the biodiversity of steinernematids and may have useful application for determining differences in pathogenicity against insect pests.

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