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# MORPHOLOGICAL AND BIOLOGICAL VARIABILITY OF *STEINERNEMA FELTIAE* (NEMATODA, STEINERNEMATIDAE) ITALIAN STRAINS (1)

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Clausi M., Troccoli A., Leone D., De Luca F., Rappazzo G., Fanelli E., Ravlic J., Tarasco E. - Morphological and biological variability of *Steinernema feltiae* (Nematoda, Steinernematidae) Italian strains.

Steinernema feltiae belongs to the feltiae-kraussei-oregonensis group, clade III, and is an ubiquitarian species of entomopathogenic nematode. It is found in all types of soil and in all types of habitat. Species identification in the entomopathogenic nematodes genera *Steinernema* is a very complex task, given the broad variability of both morphological and biological traits within populations of a single species. To accomplish this, molecular techniques have been adopted which, however, require additional knowledge. Particularly relevant would be the possibility of testing in a reliable way the variability between different populations of the same species, which might represent different strains with different biological properties. During numerous samplings in Italy, several strains of *S. feltiae* were isolated. In this paper we analyze the intraspecific variability of the main morphometric and biological analysis were useful to identify characters having significant diagnostic value, allowing to reliably discriminate among strains. Seven characters routinely computed for morphology (5 morphometrics for infective juveniles, spicula and gubernaculum shapes for males) and 2 biological performances (time to achieve adult stage, reproduction and progeny) were considered. The results showed extreme variability from both morphological and biological points of view

KEY WORDS: morphometrics, biological characterization, entomopathogenic nematode

## INTRODUCTION

The cosmopolitan species Steinernema feltiae (Filipjev, 1934) (Nematoda, Steinernematidae) is probably the most famous and widespread entomopathogenic nematode (EPN) in the world (HOMINICK et al., 1997; ADAM & NGUYEN, 2002). In Italy it was isolated from 53 localities of different habitats (CLAUSI & VINCIGUERRA, 2005; TARASCO & TRIGGIANI, 1997; TARASCO et al., 2015) and resulted as the most common EPN in the Country. The aim of this work was to continue the study of the biodiversity of Italian EPNs analyzing intraspecific variability of S. feltiae. In particular, the morphological and biological variability were studied by comparing the data of 50 S. feltiae Italian populations isolated from different habitats in the southern Italian regions of Apulia, Campania, Basilicata, Calabria and Sicily (table1).

# MATERIALS AND METHODS

The study considered a total of 50 populations of *S. feltiae* of which 28 from Apulia, 3 by 3 from Basilicata, Campania and Calabria, and 13 from Sicily.

#### MORPHOLOGICAL STUDY

Infective juveniles (IJs): Morphometric analysis of Steinernema feltiae Italian populations was processed with a non-parametric method using the Kruskal-Wallis test by ranks (Kruskal–Wallis *H* test, or one-way ANOVA on ranks - SPSS Statistics, 2019). A minimum of 20 IJs were observed for each S. feltiae population. The analysis focused on the main morphometric characters of the IJs (Body Lenght, Maximum body diameter, Anterior End-Pharynx base distance, Anterior End-Excretory Pore distance, Tail lenght). Males: A minimum of 10 males were observed for each S. feltiae population. The analysis focused on the main morphological characters of the spicules and gubernacula shapes (NGUYEN & SMART, 1996; HUNT & NGUYEN, 2016).

## **BIOLOGICAL OBSERVATIONS**

Times to reach the adult stage, reproduction and emergence of the progeny were observed and measured using the "Hanging drop" Technique (POINAR, 1975), a well-established method for examining living, unstained, very small organisms (Fig. I). Our procedure employs a glass slide with a circular concavity in the centre into which a drop of fluid, containing the 'micro-

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*Table 1 – Steinernema feltiae* Italian populations. \*In parenthesis the Province acronym \*\*Abbreviations of the Italian regions: CAM = Campania, APU = Apulia, BAS = Basilicata, CAL = Calabria, SIC = Sicily, SIC-ai = Aeolian islands, SIC-pi = Pantelleria island

Strain	*Locality	**Italian regions	Alt.	Date	Vegetation	Habitat	Soil text	Species	Accession number
1.SA1	Sammichele (BA)	APU	280	Jun 1990	Cherry	Orchard	Sandy loam	S. feltiae	n.a.
2.CO1	Corato (BA)	APU	230	Feb 1992	Wild vegetation	Uncultivated land	Silt	S. feltiae	HQ412814.1
3.GR1	Grassano (MT)	BAS	300	Nov 1995	Tomato	Field	Clay loam	S. feltiae	HQ412818.1
4.G4	Gravina (BA)	APU	380	Oct 1995	Pine	Pinewood	Silty loam	S. feltiae	n.a.
5.G8	Gravina (BA)	APU	380	Dec 1995	Pine	Pinewood	Sandy loam	S. feltiae	n.a.
							•		
6.CE2	Cerignola (FG)	APU	120	Oct 1996	Olive	Orchard	Sandy loam	S. feltiae	HQ412813.1
7.MO1	Melfi (PZ)	BAS	500	Sep 1996	Apple	Orchard	Sandy loam	S. feltiae	HQ416966.1
8.CL2	Celzi (AV)	CAM	650	Oct 1996	Pear	Orchard	Sandy loam	S. feltiae	HQ412830.1
9.MU1	Mugnano del Cardinale (AV)	CAM	550	Oct 1996	Kaki	Orchard	Clay loam	S. feltiae	HQ412841.1
10.MV1	Montevergine (AV)	CAM	700	Oct 1996	Chestnut	Broadleaf wood	Sandy loam	S. feltiae	n.a.
12.LE1	Tricase (LE)	APU	50	Oct 1997	Meadows	Grassland	Silty loam	S. feltiae	HQ412819.1
13.TE1	Terlizzi (BA)	APU	200	Feb 1998	Cherry	Orchard	Clay loam	S. feltiae	HQ412831.1
14.MF1	Martina Franca	APU	350	Mar 1998	Oak	Broadleaf	Silty loam	S. feltiae	HQ412851.1 HQ412826.1
	(TA)					wood	-	-	
15.CS6	Brindisi (BR)	APU	20	Apr 1998	Artichoke	Field	Sand	S. feltiae	HQ412815.1
16.CZ19	Giamberga (CS)	CAL	800	May 1998	Pine	Pinewood	Sandy loam	-	HQ412816.1
17.CZ23	Cecita lake (CS)	CAL	1100	May 1998	Pine	Pinewood	Sandy loam	S. feltiae	HQ412817.1
18.MA12	Chiese Rupestri Park (MT)	BAS	400	Sep 1998	Cave	Cave	Sandy loam	S. feltiae	HQ412827.1
19.G16	Gravina (BA)	APU	380	Mar 1999	Pine	Pinewood	Silty loam	S. feltiae	HQ412828.1
20.Q1	Quasano (BA)	APU	150	May 1999	Meadows	Grassland	Silty loam	S. feltiae	HQ412829.1
21.MSA3	Monte S. Angelo (FG)	APU	790	Mar 1999	Meadows	Grassland	Silt	S. feltiae	HQ412811.1
22.MSA4	Monte S. Angelo (FG)	APU	790	Nov 1999	Meadows	Grassland	Silt	S. feltiae	HQ412825.1
23.BQ1	Monte S. Angelo (FG)	APU	787	Dec 1999	Meadows	Grassland	Silty loam	S. feltiae	n.a.
24.B6	Bitonto (BA)	APU	118	Dec 1999	Olive	Orchard	Silt	S. feltiae	n.a.
25.TG4	Brindisi (BR)	APU	20	Jan 2000	Swamp	Wetland	Sandy loam	S. feltiae	HQ412824.1
26.OT2	Alimini Lake (LE)	APU	25	Apr 2000	Pine	Pinewood	Sand	S. feltiae	HQ412820.1
27.ESA	S.Alfio (CT)	SIC	530	Sep 2005	Chestnut	Broadleaf wood	Sandy loam	S. feltiae	GU599911.1
28.RC8	Aspromonte (RC)	CAL	680	Jul 2005	Oak	Broadleaf wood	Sandy loam	S. feltiae	n.a.
29.ESC2	Salto del cane	SIC	1200	Sep 2005	Chestnut	Broadleaf	Sandy loam	S. feltiae	GU599905.1 GU599907.1
30.EPP	(CT) Piano	SIC	1000	Sep 2005	Chestnut	wood Broadleaf	Sandy loam	S. feltiae	GU599907.1 GU599908.1
31.EMM1	Porcheria (CT) Mt. Monaco	SIC	820	Oct 2005	Chestnut	wood Broadleaf	Sandy loam	S. feltiae	GU599904.1
32.EPC	(CT) Pietracannone	SIC	1000	Oct 2005	Chestnut	wood Broadleaf	Sandy loam	S. feltiae	GU599910.1 GU599912.1
33.ETA	(CT) Tarderia (CT)	SIC	750	Oct 2005	Chestnut	wood Broadleaf	Sandy loam	S. feltiae	GU599906.1
34.EMA	Triciala (CT)	SIC	950	Oct 2005	Chestnut	wood Broadleaf	Sandy loam	S. feltiae	GU599909.1 GU599913.1
35.OT9	Otranto (LE)	APU	20	May 2006	Oak	wood Broadleaf wood	Sandy loam	S. feltiae	HQ416967.1
26 OT10	Otroate (LE)		20	Mar. 2006	Dina		Sand	C falting	
36.OT10	Otranto (LE)	APU	20	May 2006	Pine	Pinewood	Sand	S. feltiae	n.a.
37.OT11	Otranto (LE)	APU	20	May 2006	Pasture	Grassland	Sand	S. feltiae	HQ412821.1
38.OT14	Otranto (LE)	APU	20	Jun 2006	Pine	Pinewood	Sand	S. feltiae	HQ412822.1
39.OT15	Otranto (LE)	APU	20	Jun 2006	Artichoke	Field	Sand	S. feltiae	HQ412823.1
40.M31	Canale di Pirro (BR)	APU	300	May 2007	Wheat	Field	Silty loam	S. feltiae	n.a.

Strain	*Locality	**Italian regions	Alt.	Date	Vegetation	Habitat	Soil text	Species	Accession number
41.M23	Pianelle Park (TA)	APU	200	May 2007	Oak	Broadleaf wood	Sandy loam	S. feltiae	n.a.
42.M50	Canale di Pirro (TA)	APU	300	May 2007	Artichoke	Filed	Sandy loam	S. feltiae	n.a.
43.M62	Galeone Forest (TA)	APU	300	Jun 2007	Oak	Broadleaf wood	Sandy loam	S. feltiae	n.a.
44.FF1	Francavilla Fontana (BR)	APU	150	Sep 2008	Wheat	Field	Clay loam	S. feltiae	n.a.
45.MUE3	Pantelleria (TP)	SIC-pi	50	Jun 2010	Mulberry	Broadleaf wood	Sandy loam	S. feltiae	n.a.
46.PR4	Gornalunga Lake (CT)	SIC	800	Nov 2010	Pine	Pinewood	Sandy loam	S. feltiae	n.a.
47.CTSA18	San Leonardo River (SR)	SIC	5	Dec 2008	Eucalyptus	Pinewood	Sand	S. feltiae	HQ412832.1
48.CT27	Mt. Serra (CT)	SIC	450	Apr 2009	Oak	Broadleaf wood	Sandy loam	S. feltiae	HQ412834.1
49.VE1	Oasi di Vendicari (SR)	SIC	23	May 2009	Olive	Orchard	Clay loam	S. feltiae	HQ412835.1
50.SAL3	Salina (ME)	SIC-ai	130	Sep 2009	Pine	Pinewood	Sandy loam	S. feltiae	HQ412836.1

organisms', hangs from a coverslip. The hemolymph was taken from the *Galleria mellonella* L. (Lepidoptera, Pyralidae) larva: the middle legs of the insect were cut and 2 drops of hemolymph coming out were placed on the square coverslip ( $20 \times 20$  mm). Meanwhile, the

entomopathogenic nematodes from different populations of *S. feltiae* were kept in beakers (a beaker for each different population). Later, a few nematodes (4-6 IJs) were taken with a thin needle to another beaker containing hyamine solution (Hyamine 1622 solution

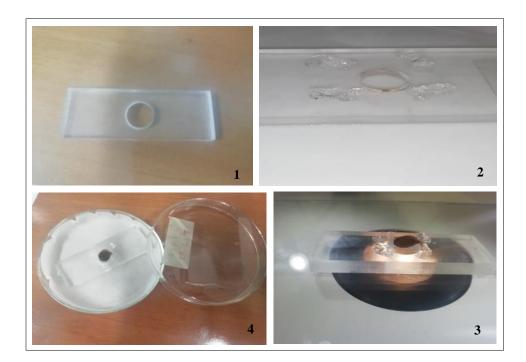


Fig. I – Biological observations - "Hanging drop" technique: 1. Perforated slide; 2. Perforated slide with gel to keep the cover slide with the haemolymph drop; 3. Petri dish with humid filter paper and slide with haemolymph drop; 4. slide with haemolymph drop under microscope for observation.

	an strai	s All-Pairwise Comparis ns			
Nema	Mean	Homogeneous Groups	Nema Me	ean B	Homogeneous Groups
4	933.90	A	19 464	4.03	DEFGHIJKLMN
9	896.50	AB	16 45	5.65	EFGHIJKLMN
7	844.15	ABC	10 448	8.07	EFGHIJKLMN
24	838.20	ABCD	36 43	9.90	EFGHIJKLMN
3	805.67	ABCDE	38 43	7.25	EFGHIJKLMN
35	780.75	ABCDEF	12 43	4.75	EFGHIJKLMN
44	780.75	ABCDEF	32 433	1.05	EFGHIJKLMN
47	780.75	ABCDEF	27 41:	1.90	FGHIJKLMN
42	772.30	ABCDEFG	34 402	2.00	GHIJKLMN
28	740.92	ABCDEFGH	8 393	3.18	HIJKLMN
22	719.78	ABCDEFGHI	6 378	8.50	HIJKLMN
45	719.78	ABCDEFGHI	23 36:	1.52	IJKLMN
21	698.85	ABCDEFGHI	39 350	0.60	IJKLMN
29	640.10	ABCDEFGHIJ	33 312	2.20	JKLMN
46	640.10	ABCDEFGHIJ	30 283	3.18	JKLMN
43	638.83	ABCDEFGHIJ	17 23	7.00	KLMN
26	634.25	ABCDEFGHIJ	49 23	7.00	KLMN
13	619.58	ABCDEFGHIJ	15 223	1.20	KLMN
31	595.20	ABCDEFGHIJK	15 22: 40 21: 14 21: 2 20: 41 14: 48 14:	8.20	LMN
1		ABCDEFGHIJKL	14 21	6.20	LMN
11	549.75	BCDEFGHIJKL	2 201	8.68	LMN
20	542.95	BCDEFGHIJKL	41 14:	3.45	MN
18	490.58	CDEFGHIJKLM	48 14	3.45	MN
5	486.35	CDEFGHIJKLM	37 94	. 650	N
25	475.28	CDEFGHIJKLM	50 94	.650	N
					200 µm

Fig. II - Variability of Body Length (BL): homogeneous morphometrics in comparison for 50 strains of Italian *S. feltiae*. For strain number see Table 1.

31.73 21.57 98.75 91.07 65.20 36.10 28.85 15.75 98.05	A AB AB ABC ABCD ABCD	35 44 47 25 23 19	494.35 494.35 493.03 486.58	Homogeneous Groups ABCDEFGHIJK ABCDEFGHIJK ABCDEFGHIJK ABCDEFGHIJK
21.57 98.75 91.07 65.20 36.10 28.85 15.75 98.05	A AB AB ABC ABCD ABCD	44 47 25 23 19	494.35 494.35 493.03 486.58	ABCDEFGHIJK ABCDEFGHIJK ABCDEFGHIJK ABCDEFGHIJK
98.75 91.07 65.20 36.10 28.85 15.75 98.05	AB AB ABC ABCD ABCD	47 25 23 19	494.35 493.03 486.58	ABCDEFGHIJK ABCDEFGHIJK
91.07 65.20 36.10 28.85 15.75 98.05	AB ABC ABCD ABCD	25 23 19	493.03 486.58	ABCDEFGHIJK
65.20 36.10 28.85 15.75 98.05	ABC ABCD ABCD	23 19	486.58	
36.10 28.85 15.75 98.05	ABCD	19		ADODDEGUT TV
28.85 15.75 98.05	ABCD			ABCDEFGHIJK
15.75 98.05			442.85	BCDEFGHIJK
98.05	ABCD	18	426.78	BCDEFGHIJK
	ADOD	8	409.75	CDEFGHIJK
		16	394.10	CDEFGHIJKL
	ABCDE	36	388.70	DEFGHIJKLM
	ABCDEF	14	383.37	DEFGHIJKLM
		12	336.95	EFGHIJKLM
		17	319.95	FGHIJKLM
		49	319.95	FGHIJKLM
		38	298.50	FGHIJKLM
		33	292.10	GHIJKLM
		34	279.20	HIJKLM
		37	270.85	IJKLM
		50	270.85	IJKLM
		15	240.45	JKLM
		41	226.95	KLM
		48	226.95	KLM
		32	202.00	KLM
		1	27.050	LM
26.95	ABCDEFGHIJK	2	14.000	М
554422107755	0.00 9.03 9.03 6.60 0.50 6.93 1.97 2.95 2.20 1.97 6.63	6.93 ABCDEFGHI 1.97 ABCDEFGHIJ 2.95 ABCDEFGHIJK 2.20 ABCDEFGHIJK 1.97 ABCDEFGHIJK 6.63 ABCDEFGHIJK	0.00 ABCDEFG 17   0.00 ABCDEFG 49   9.03 ABCDEFGH 38   9.03 ABCDEFGH 33   6.60 ABCDEFGHI 34   0.50 ABCDEFGHI 37   6.93 ABCDEFGHI 50   1.97 ABCDEFGHIJK 41   2.20 ABCDEFGHIJK 48   1.97 ABCDEFGHIJK 32   6.63 ABCDEFGHIJK 1	0.00 ABCDEFG 17 319.95   0.00 ABCDEFG 49 319.95   9.03 ABCDEFGH 38 298.50   9.03 ABCDEFGH 33 292.10   6.60 ABCDEFGHI 34 279.20   0.50 ABCDEFGHI 37 270.85   6.93 ABCDEFGHIJ 50 270.85   2.95 ABCDEFGHIJK 41 226.95   2.20 ABCDEFGHIJK 48 226.95   1.97 ABCDEFGHIJK 32 202.00   6.63 ABCDEFGHIJK 1 27.050

ema	Mean	Homogeneous Groups	Nema	Mean	Homogeneous Groups	
40	988.00	A	31	487.28	CDEFGHIJ	
30	898.50	AB	10	473.08	CDEFGHIJ	
9	777.08	ABC	12	470.25	CDEFGHIJ	
35	776.35	ABC	36	453.15	CDEFGHIJ	
44	776.35	ABC	24	451.95	CDEFGHIJ	-
47	776.35	ABC	33	443.65	CDEFGHIJ	- and
22	757.73	ABCD	20	438.98	CDEFGHIJ	
45	757.73	ABCD	32	437.45	CDEFGHIJ	1
7	751.00	ABCDE	23	405.88	CDEFGHIJ	
27	748.92	ABCDE	38	386.40	DEFGHIJK	
3	737.47	ABCDE	25	378.15	EFGHIJK	
8	720.42	ABCDE	18	309.15	FGHIJK	
4	653.08	ABCDEF	41	303.45	FGHIJK	
29	653.08	ABCDEF	48	303.45	FGHIJK	
46	653.08	ABCDEF	39	294.10	FGHIJK	
28		ABCDEFG	16	284.00	FGHIJK	
43	602.48	BCDEFGH	6	258.45	GHIJK	
21	600.78	BCDEFGH	37	235.05	HIJK	
26	575.53	BCDEFGHI	50	235.05	HIJK	
13	564.25	BCDEFGHI	17	229.25	HIJK	
11	552.75		49	229.25	HIJK	
19	544.70		14	207.25	IJK	
5	541.83		15	164.03	JK	
42			1	26.925	ĸ	
34	508.45	CDEFGHIJ	2	14.075	ĸ	

Fig. III –Variability of Maximum Body Diameter: homogeneous morphometrics in comparison for 50 strains of Italian *S. feltiae.* For strain number see Table 1.

Fig. IV –Variability of Head-excretory pore distance: homogeneous morphometric's groups in comparison for 50 strains of Italian *S. feltiae*. For strain number see Table 1.

L GDAL		s All-Pairwise Compari	······································
Iema	Mean	Homogeneous Groups	Nema Mean Homogeneous Groups
21	942.65	A	25 513.58 CDEFGHIJ
4	914.25	AB	22 507.85 CDEFGHIJ
35	881.60	ABC	45 507.85 CDEFCHIJ
44	881.60	ABC	11 496.75 DEFGHIJK
47	881.60	ABC	10 476.10 DEFGHIJKL
24	825.28	ABCD	34 467.65 DEFGHIJKL
28	822.13	ABCD	6 442.15 EFGHIJKL
13	732.50	ABCDE	16 441.13 EFGHIJKL
33	725.20	ABCDEF	36 431.90 EFGHIJKL
8	706.08	ABCDEF	26 417.10 EFGHIJKL
3	703.65	ABCDEF	41 351.45 FGHIJKLM
43	675.40	ABCDEFG	48 351.45 FGHIJKLM
42	656.65	ABCDEFG	39 826.80 GHIJKLM
7	656.00	ABCDEFG	17 311.73 GHIJKLM
29	631.10	ABCDEFG	49 311.73 GHIJKLM
46	631.10	ABCDEFG	32 252.20 HIJKLM
27	597.65	ABCDEFGH	14 224.90 HIJKLM
5	596.60	ABCDEFGH	40 201.15 IJKLM
31	585.02	ABCDEFGH	18 198.28 IJKLM
23	558.28	BCDEFGHI	15/147.13 JKLM
	552.90	and the second s	37 129.65 KLM
12	524.67	CDEFGHI	50 129.65 KLM
20	517.05	CDEFGHIJ	30 116.35 LM
19	515.48	CDEFGHIJ	1 28.600 M
9	515.08	CDEFGHIJ	2 12.400 M
2		ELECTRONIC STATES	50 µm

Fig. V – Variability of Head-pharynx base distance: homogeneous morphometric's groups in comparison for 50 strains
of Italian S. feltiae. For strain number see Table 1.

Nema	Mean	Homogeneous Groups	Nem	a Mean	Homogeneous	Groups
9	915.33	A	20	530.23	CDEFGHI	
7	908.03	AB	26	517.90	CDEFGHI	
35	833.05	ABC	11	514.85	CDEFGHI	
44	833.05	ABC	10	504.73	CDEFGHI	
47	833.05	ABC	38	504.25	CDEFGHI	
4	760.15	ABCD	16	499.83	CDEFGHI	
3	745.55	ABCD	25	478.37	CDEFGHI	
28	708.33	ABCDE	36	463.75	CDEFGHIJ	
29	704.50	ABCDE	40	432.70	DEFGHIJ	Summer Man
46	704.50	ABCDE	24	428.40	DEFGHIJ	
39	681.40	ABCDEF	12	406.70	DEFGHIJ	2 States and States
21	664.38	ABCDEF	27	356.30	EFGHIJK	
42	643.70	ABCDEFG	15	340.93	EFGHIJK	State of the state of the
5	628.05	ABCDEFG	37	322.00	FGHIJK	
8	598.98	ABCDEFGH	50	322.00	FGHIJK	
31	588.03	ABCDEFGH	41	287.55	GHIJK	
43	568.00	ABCDEFGHI	48	287.55	GHIJK	
23	564.45	ABCDEFGHI	14	237.40	HIJK	
13	561.23	ABCDEFGHI	6	225.45	HIJK	
22	561.18	ABCDEFGHI	17	203.25	IJK	
45	561.18	ABCDEFGHI	49	203.25	IJK	
34	557.70	ABCDEFGHI	30	99.875	JK	
32	535.60	BCDEFGHI	18	94.250	JK	
19	532.28	CDEFGHI	1	22.925	K	
33	530.70	CDEFGHI	2	18.225	K	
						20 µm

Fig. VI - Variability of Tail lenght: homogeneous morphometric's groups in comparison for 50 strains of Italian *S. feltiae*. For strain number see Table 1.

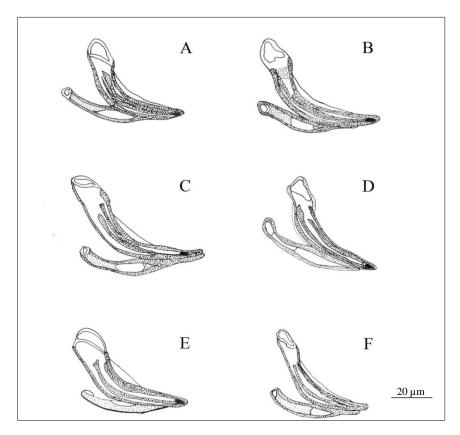


Fig. VII - Morphology of spicules and gubernacula of Italian *Steinernema feltiae* strains: 6 different typologies were recognized.

0.004M), for 5" for disinfection of the nematodes. Then the disinfected nematodes were taken carefully with a needle and placed on the coverslip inside the drop of hemolymph. The slide with the hole in the middle was used for easier and clearer observation of the nematodes. A gel suspension was settled on the four corners of the coverslip around the hole to keep the coverslip apart from the slide. The next step was to carefully invert the coverslip, trying not to disturb the hemolymph drop with nematodes inside, and mount it exactly above the hole situated in the middle of the slide. The hole in the middle of the slide was used to keep the droplet intact and to facilitate viewing under the microscope (Fig. I). Then the slide was placed inside a Petri dish containing a wet filter paper to maintain the required moisture. Water was added in the Petri dishes every two days to keep a saturated level of moisture to prevent the drops of hemolymph from dehydration. Petri dishes with slides were kept in the dark at 24 °C. The aim of this method was to monitor the nematode development and mating inside the hemolymph drops. The emergence of the new offsprings was also observed.

## RESULTS

With the morphometric analysis we highlighted different major groupings of the EPN. Within the variability of the populations, some showed interesting morphometric similarities and there were groups showing homogeneous morphometrics in comparison to the others. For Body Length (BL) there were up to 14 similarity degrees with a maximum of 28 populations in the same homogenous group (Fig. II); for Maximum Body Diameter (MBD) there were 13 similarity degrees with up to 31 populations in the same homogenous group (Fig. III); for Head-Excretory pore (He) 11 similarity degrees were observed with up to 32 populations in the same homogenous group (Fig. IV); for Head-Pharynx base distance (H-P) the similarity degrees were 13 with up to 29 populations in the same homogenous group (Fig. V). Finally, for Tail Length (TL) 11 similarity degrees were observed with up to 31 populations in the same homogenous group (Fig. VI). Spicule morphology of S. feltiae isolates showed some differences, basically attributable to at least 6 typologies

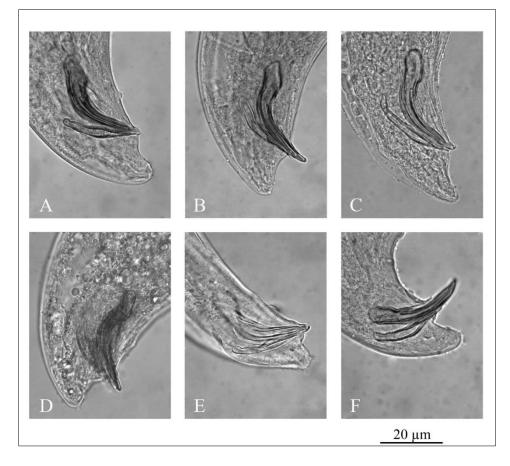


Fig. VIII – Morphology of spicules and gubernacula of Italian *Steinernema feltiae* strains: 6 different typologies were recognized.

(Figs. VII-VIII). Most of the isolates belonged to the A, B (i.e. population n. 32 EPC and 27 ESA, as A-type and B-type respectively) and C (i.e. population n. 29 ESC2) type showing larger spicules with respect to all other isolates, with head longer than wide. Isolates of F-type (i.e. population n. 30 EPP) also showed a head longer than wide, with a thin velum extending almost to the entire length of the blade. In isolates of D-type (i.e.

population n. 31 EMM1) and A-type (i.e. population n. 32 EPC) the head of spicule was as long as wide. The velum was thin and as long as spicule blade, in A, B and F (isolates n. 32 EPC, n. 27 ESA and n. 30 EPP), whereas it was short (about half of blade length) in C and E isolates (n. 29 ESC2 and n. 2 CO1, C-type and Etype respectively). In isolates of D-type (i.e. n. 31 EMM1) the velum was hardly visible. A slightly pronounced rostrum could be observed in isolates of types C and D, being nearly absent in all other spicule typologies. Finally, differences were observed in gubernaculum shape, with the anterior end generally in axis with corpus, except in isolates of C-type which showed an obtuse angle. The first group, common to most of the isolates, presented a slender spicule, with head and foil long and thin, while the second group showed spicules stubbier in all the parts (Figs. VII-VIII).

BIOLOGICAL OBSERVATIONS - Both sexes were obtained in every samples. The adult stages were found from one day (3 populations) to 1.5 day (5 populations), 2 days (12 populations), 2.5 days (20 populations) and 3 days (10 populations). Mating took place shortly after reaching the adult stage (for the majority of the strains two-three days after they were put into the hemolymph drop). The progeny emerged after 4 to 7 days from mating. The nematode specimens were observed on the edge of the hemolymph drop.

# DISCUSSION AND CONCLUSIONS

Our study focused on the intraspecific characteristics of 50 populations isolated in Italy and the results showed extreme variability from both morphological and biological points of view. Paraphrasing the original sentence of Aaron Levenstein, related to the statiscts, with reference to the morphological and biological aspects of the nematodes, we could say that "Molecular analysis are like bikinis: what they reveal is suggestive, but what they conceal is vital". Molecular analyses have now become an indispensable tool for species identification, but the results of our survey show that morphological and biological studies on the variability of individual populations of *S. feltiae* are equally important and provide fundamental data for the characterisation of the species. Morpho-biological and molecular studies are functional to each other and must proceed in parallel for a correct and complete species characterization.

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