

**Occurrence of *Steinernema apuliae* in Southern Italy and its infectivity in relation to temperatures**



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# “Long Nematodes” strains from Italy

- **Surveys** on the occurrence of entomopathogenic nematodes carried out in Southern Italy during the past 10 years pointed out the presence of numerous species and strains of EPNs in different biotopes.
- **Among** these Italian EPNs, 10 strains belonging to the “Long Nematodes” (*Steinernema glaseri* Group) were collected.
- **LONG NEMATODES:** IJs of the EPN strains belonging to this group have some **morphological peculiarities**, such as: body length of 840-1305  $\mu\text{m}$ , tail length of 66-81  $\mu\text{m}$  and the distance between the excretory-pore and the head that is 57-67% of the oesophagus length, whereas the differences among the populations of the same group are related to the shape of the cephalic region and the tail of IJs, and the shape of the male spicule

## *Steinernema glaseri* Group

*S. glaseri* (Steiner, 1929)

*S. arenarium* (Artyukhovsky, 1967)

*S. caudatum* Xu, Wang & Li, 1991

*S. longicaudum* Shen & Wang, 1992

*S. cubanum* Mracek, Hernandez & Boemare, 1994

*S. puertoricense* Roman & Figueroa, 1994

*S. kari* Waturu, Hunt & Reid, 1997

*S. ohioense* Lucskai & Klein in Lucskai, 1999

*S. loci* Phan, Nguyen & Moens, 2001

*S. thanhi* Phan, Nguyen & Moens, 2001

*S. apuliae* Triggiani, Mracek & Reid, 2004

# Reports of LNs from Europe

- *S. arenarium* (syn. *S. anomali*) was the first LN isolated in Europe, described in **Central Russia** in 1967 (Artyukhovsky, 1967) and re-described from the type locality in 1997 (Artyukhovsky *et al.*, 1997).
- Afterwards other LN strains identified as *S. arenarium* (or *S. anomali*), *S. glaseri* or as “*glaseri*-like”, were collected in **Poland** (Tomalak, 2002), **Czech Republic** (Mráček *et al.*, 1999), **Slovak Republic** (Sturhan and Lišková, 1999), **Switzerland** (Kramer *et al.*, 2001; Spiridonov *et al.*, 2002), **France** (Sturhan and Mráček, 2002), **Spain** (De Doucet and Gabarra, 1994; García del Pino and Palomo, 1995), **Portugal**, Azores (Rosa *et al.*, 2000) and **Italy** (Tarasco and Triggiani, 1997; Triggiani and Tarasco, 2002).
- In the Southern and Eastern Mediterranean area the occurrence of members of this Group has been reported in **Morocco** (Peters, pers. comm.) and **Palestine** (Sansour *et al.*, 2003)

# The Survey in Southern Italy



A total of more than 800 locations were investigated in the vicinity of Apulia, Calabria, Basilicata, Molise, Campania and Sardinia Regions

Final instars of *Galleria mellonella* were used as bait insects to trap the nematodes



Long handle tea infuser used for the *Galleria mellonella* baiting technique

**White traps** to collect the IJs

the progeny were used for  
**identification**

Morphological  
characters of IJs  
and males



Interbreeding with  
original species



Molecular characterisation by  
RFLP analysis of the ITS region  
of the ribosomal DNA repeat unit

EPNs were collected from **68** locations

**8.5%** of the total soil samples collected

The nematodes were identified as

*Steinernema feltiae* - 27 strains (3.4% of the total)

*S. affine* - 10 strains (1.3%)

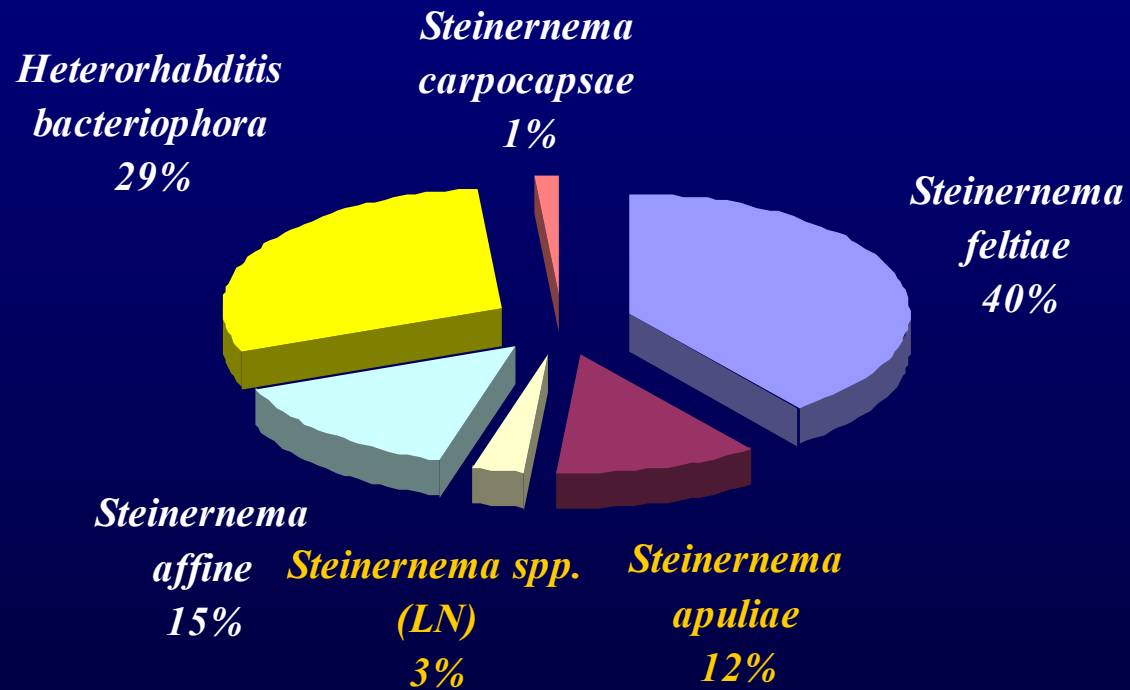
*S. carpocapsae* - 1 strain

***S. apuliae* – 8 strains (1%)**

*Heterorhabditis bacteriophora* - 20 strain (2.5%)

***Steinernema* spp. (LN) – 2 strains**

# EPN species (%) isolated in Southern Italy



Geographical  
distribution of  
LN strains in  
Southern Italy ●



# Characteristics of the sites with LN strains isolated in Southern Italy

Strain	Locality	Altitude	Time	Habitat	Soil texture	pH	Org. Cont.
ItS-C15*	Castellaneta (TA)	0	Sep'96	Sea coast	Sand	7.4	0.4
<b>ItS-MS10</b>	<b>Margherita di S. (BA)</b>	<b>30</b>	<b>Oct'96</b>	<b>Salt pan</b>	<b>Silt</b>	<b>7.9</b>	<b>1.15</b>
<b>ItS-MS3</b>	<b>Margherita di S. (BA)</b>	<b>30</b>	<b>Oct'96</b>	<b>Uncultivated</b>	<b>Sand</b>	<b>7.4</b>	<b>0.27</b>
<b>ItS-LD3</b>	<b>Metaponto (MT)</b>	<b>20</b>	<b>Oct'96</b>	<b>Pinewood</b>	<b>Sand</b>	<b>7.9</b>	<b>0.34</b>
<b>ItS-LE13</b>	<b>Torre Pali (LE)</b>	<b>0</b>	<b>Oct'97</b>	<b>Sea coast</b>	<b>Sand</b>	<b>8.2</b>	<b>0.3</b>
<b>ItS-ZA11</b>	<b>Zapponeta (FG)</b>	<b>30</b>	<b>Nov'97</b>	<b>Uncultivated</b>	<b>Sand</b>	<b>7.8</b>	<b>0.27</b>
<b>ItS-CS3</b>	<b>Brindisi (BR)</b>	<b>20</b>	<b>Apr'98</b>	<b>Tamarisk</b>	<b>Sand</b>	<b>8.3</b>	<b>0.11</b>
<b>ItS-TA14</b>	<b>Taranto (TA)</b>	<b>0</b>	<b>Sep'98</b>	<b>Sea coast</b>	<b>Sand</b>	<b>8.3</b>	<b>0.01</b>
ItS-C31	Castellaneta (TA)	20	May 00	Pinewood	Sand	8.0	3.8
<b>ItS-TC5</b>	<b>III Cavone (MT)</b>	<b>0</b>	<b>Mar'01</b>	<b>Sea coast</b>	<b>Sand</b>	<b>7.8</b>	<b>0.27</b>

These data show that the LNs occurrence in Southern Italian biotopes depends on the **habitat** and the **characteristics of the soil**

Italian LNs were isolated only along the coast and prefer sandy soils; only 1 strain (ItS-MS10) was collected along a salt pan border from a silty soil.

The number of **10 LN strains** isolated in Southern Italy is higher in comparison to the LN strains isolated in other Countries (Spain, Czech Republic, Slovak Republic, Poland, Russia, Portugal, Switzerland, France, Palestine, Morocco)

**Proposal:** a research project on the EPNs (in particular Long Nematodes) of the Mediterranean Basin

## Biological characterization of *S. apuliae*

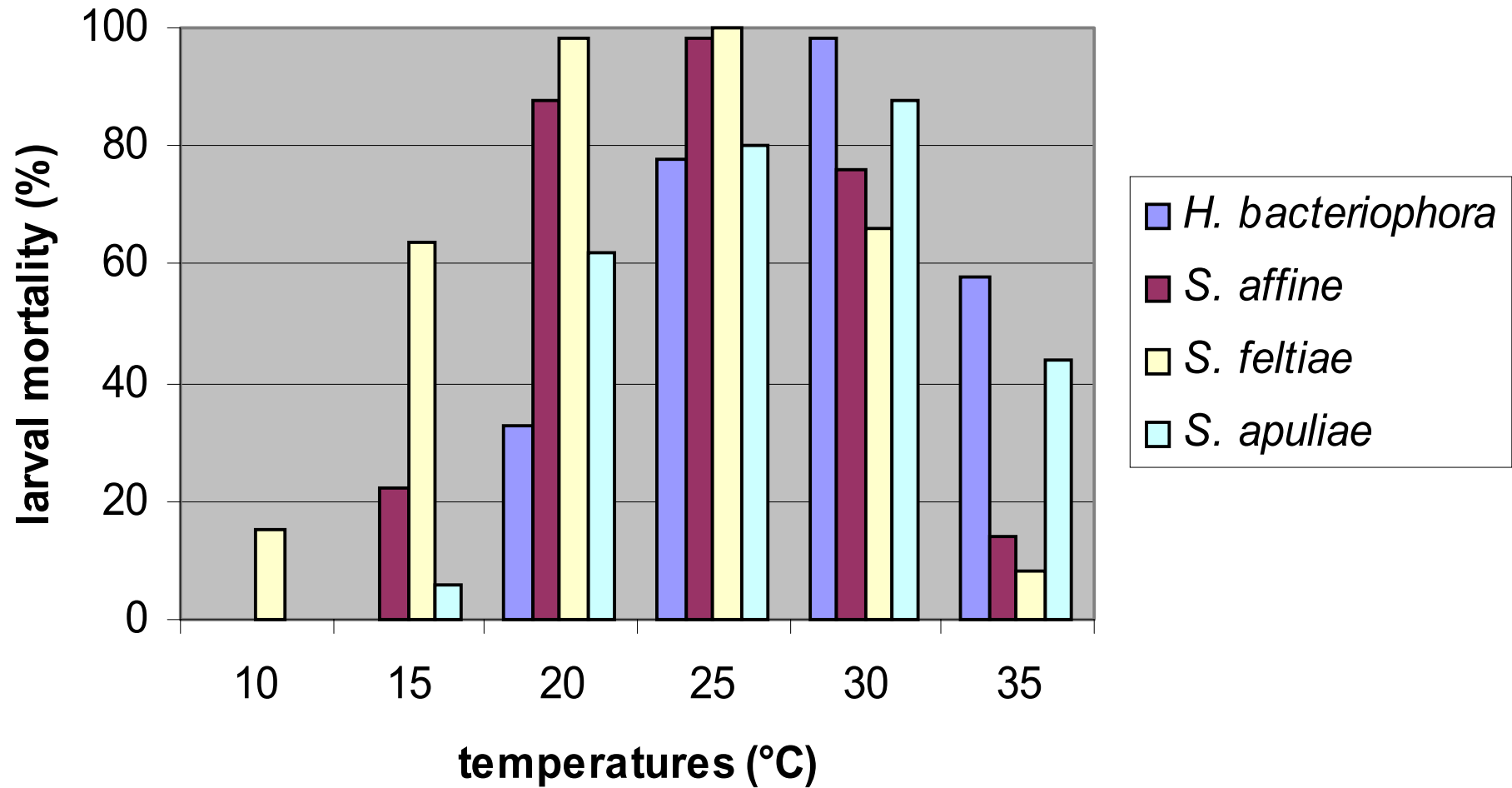
The biological characterization of this new species has been started with the evaluation of its infectious activity against *Galleria mellonella* L. (Lepidoptera, Galleriidae) larvae in relation to temperature.

The infectivity was determined by 2 larval mortality rate assays in relation to different temperature values for comparing *S. apuliae* and other 3 EPN species (*S. feltiae*, *S. affine*, *H. bacteriophora*, italian strains)

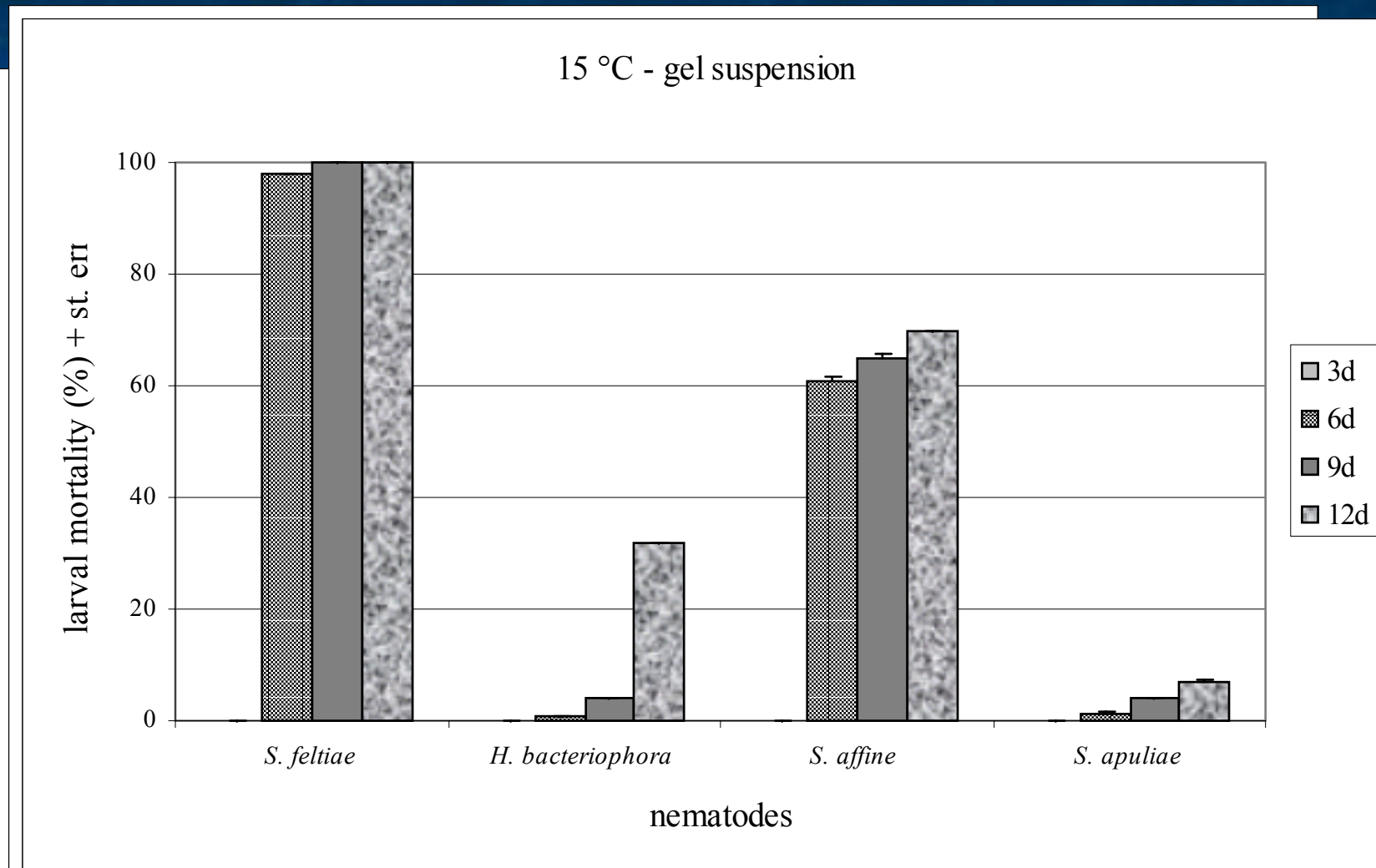
In the **First experiment** the percentage of larval mortality was recorded after a 72-hr exposure period to the EPNs IJs at 6 temperatures between 10 and 35°C, at intervals of 5°C, using IJs in aqueous suspension.

The **Second experiment** was performed to compare the infectivity of the same strains of nematodes at 5°C, 10°C and 15°C, testing IJs in a gel suspension (Idrosorb SR 202).

# first experiment – the results



## second experiment – the results



IJs in water suspension killed the *Galleria* larvae quicker than those in the gel but the gel suspension kept nematodes alive and active longer

# And continuing with the biological characterization



- Application of *S. apuliae* IJs to different hosts
  - Lepidoptera (*G. mellonella* and *T. pityocampa* larvae),  
Diptera (*B. oleae* larvae and pupae), Coleoptera (*B. elephas* and *C. tenebrionis* larvae) and , Rynchota (*C. ciliata* and *M. unicostata*)
- Tolerance tests
  - UV, desiccation
- Storage bioassays

