



EPNs and *Bursaphelenchus* spp. in CREA collection

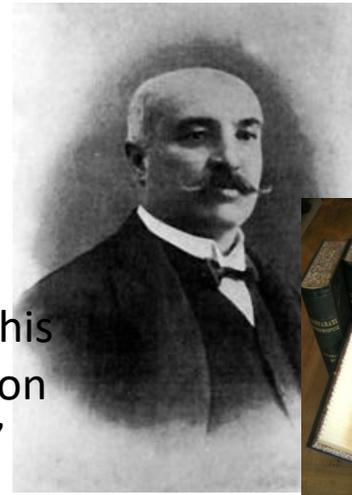
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Roversi



The origin



In 1875, the “Stazione di Entomologia Agraria” was established in Florence by Specola in order to control plant pest. The first director was Adolfo Targioni Tozzetti.



1902-1927, Antonio Berlese was the director of this structure. He created an important mites collection and established the “REDIA – Journal of Zoology”

In 1967, the Station changed name into “Istituto Sperimentale per la Zoologia Agraria” and the Section of Nematology was established.

The new building was inaugurated in 1976.



The present

CREA is the leading Italian research organization devoted to the food industry, supervised by Mipaaf. It has scientific expertise in the agricultural, fisheries, forestry, nutrition and socio-economic sectors.

CREA is organized in 12 Centres distribute in the whole national territory.



CREA – DC Plant Protection and Certification

The Center is addressed to plant protection in agricultural and forestry by biotic and abiotic agents.

The Centre defines sustainable, integrated and biological control methods of the agro-ecosystem.

It is a national reference for control and certification of pre-multiplication materials.





CLSM
Confocal Laser Scanning Microscope



TEM
Transmission Electron
Microscope



SEM
Scanning Electron Microscope



Liquid nitrogen freezer at -196°C



Liquid Nitrogen Storage Dewar



Mechanical freezer at -140°C



Mechanical freezer at -80°C



**DSC
Differential Scanning Calorimetry**

Nematode collections

- **The nematode collection has been established by Dr. Anna Marinari Palmisano since 1967; today the collection is implemented.**
- **The collection is mainly done through fieldwork relating to active research programmes.**
- **Collection is arranged in systematic order where possible.**

Type of collection		Species	Species identification
Collection of dead specimens	Mounted on slide	Plant parasitic nematodes Entomopathogenic nematodes	Morphological identification Morphological and molecular identification
	Tubes	Plant parasitic nematodes	Morphological identification
	Wet-preserved in formalin	Part of plant damage by nematodes	
Collection of live specimens	Live population stored in climatic chambers	Plant parasitic nematodes Entomopathogenic nematodes	Morphological and molecular identification
	Cryoconserved specimens	Plant parasitic nematodes Entomopathogenic nematodes	Morphological and molecular identification

Collection of dead specimens mounted on slide – Plant parasitic nematodes

A total of 2033 slides belonging to Plant parasitic nematodes have been reared since 1967.

Main Families represented:

Anguinidae
Aphelenchidae
Aphelenchoidae
Criconematidae
Dolichodoridae
Heteroderidae
Hoplolaimidae
Meloidogynidae
Longidoridae
Parasitaphelenchidae
Pratylenchidae
Tylenchidae
Tylenchulidae

Main plant origin:

Cereals
Vegetables
Fruit trees
Ornamental plants
Forest trees

Main Country of origin:

Italy
Austria
Portugal
Germany
Japan
China
Somalia



This collection needs to be digitized to be more enjoyable.



Collection of plant parasitic nematode stored in formalin

A total of 3200 specimens are stored in formalin (4%)

Tubes with plant parasitic nematodes



Part of plant damaged by nematodes



Collection of dead specimens mounted on slide – Entomopathogenic nematodes

An entomopathogenic nematodes collection has been recently established.

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Oscheius onirici sp. n. (Nematoda: Rhabditidae): a new entomopathogenic nematode from an Italian cave

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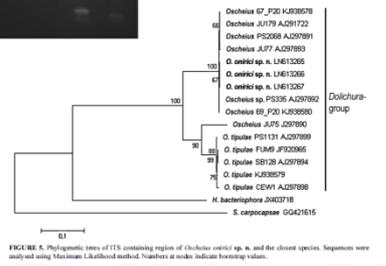
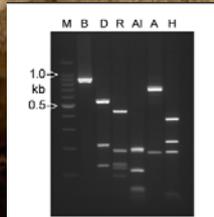
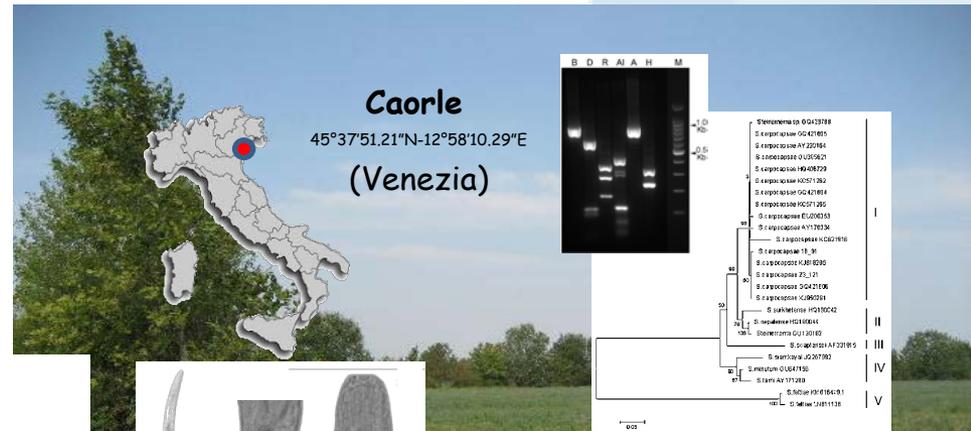


FIGURE 5. Phylogenetic trees of ITS containing region of *Oscheius onirici* sp. n. and the closest species. Sequences were analyzed using Maximum Likelihood method. Numbers at nodes indicate bootstrap values.



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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION
OF A *STEINERNEMA CARPOCAPSAE* (NEMATODA STEINERNEMATIDAE)
STRAIN ISOLATED IN VENETO REGION (ITALY)

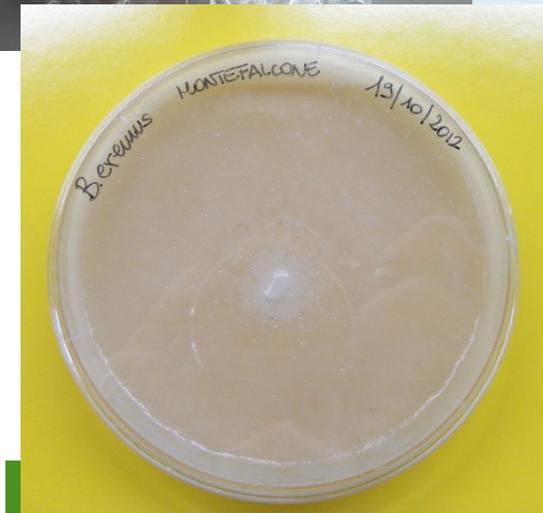
Steinernema carpocapsae
(ItS-CAO1)

Bursaphelenchus spp.

Many species belonging to *Bursaphelenchus* genus are cultured in Petri dishes on *Botrytis cinerea* fungus. Dextrose substrate with add of glycerol (5% v/v) is uses. The Petri dishes are stored at 8-9°C for 45 days. After this period the population has to be cultured again on fresh fungus.

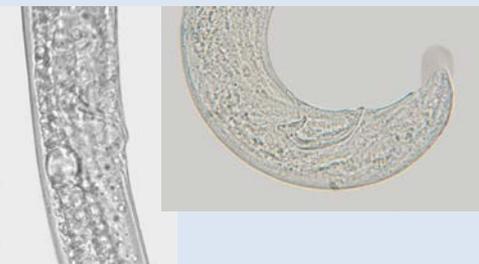
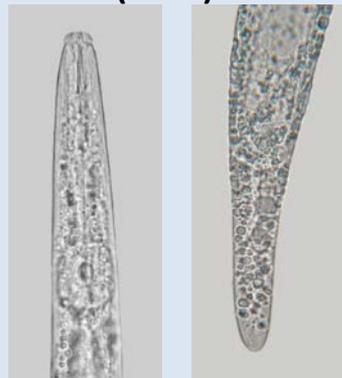


Currently, the live collection consists of 35 populations of different species of *Bursaphelenchus*.



B. mucronatus, *B. fraudulentus*, *B. eremus*, *B. xylophilus*, *B. minutus* are present in the Mediterranean Region

***B. xylophilus* Steiner and Buher (1934)**



B. xylophilus: 1 femmina, estremità anteriore; 2 femmina, cada arrotondata; 3 femmina, flap; 4 maschio, estremità posteriore, spicole e bursa (Foto B. Carletti).

Species	COD	Plant/animal origin	Country of origin
<i>B. mucronatus</i>	IT4 IT5 IT7 IT13 IT16 UA-ITI IT 38 C60/11 RU-IT1 F2 VR473 IT12 IT39	Coniferous wood Coniferous wood Coniferous wood Coniferous wood Coniferous wood Coniferous wood Coniferous wood Vineyard wood Larch wood Coniferous wood Coniferous wood Insect Insect	Italy Italy Italy Italy Italy Italy Italy Portugal Russia France Austria Italy Italy
<i>B. thailandae</i>	UN RC-A RC-A VR448 RC-AT8	Coniferous wood Coniferous wood Coniferous wood Coniferous wood	Japan Unknown Unknown Unknown
<i>B. fraudulentus</i>	IT23 IT34 IT43	Oak wood Oak wood Insect on Oak trees	Italy Italy Italy
<i>B. eremus</i>	IT17 IT18 IT19 IT35 IT36 IT20 IT21 IT22 IT37 DE39 IT45	Oak wood Oak wood Coniferous wood	Italy Italy Italy Italy Italy Italy Italy Italy Italy Italy German Italy
<i>B. xylophilus</i>	DE1	Coniferous wood	Portugal
<i>B. minutus</i>	CREA-ABP1	Insect on coniferous	Italy (2016)
<i>Bursaphelenchus</i> sp.*	ADCCO161018-7	Coniferous wood	Italy
<i>Bursaphelenchus</i> sp.*		Iroko wood	Camerun

*Molecular determination no concluded

** Morphological and molecular determination no concluded

Entomopathogenic nematodes



The presence of entomopathogenic nematodes (EPNs) is assessed using the ***Galleria* bait method**. The soil samples are placed in plastic containers and one steel mesh pocket containing two last larval instars of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) is placed in each container and kept at room temperature ($20\pm 3^\circ\text{C}$).



Cadaver of larvae with nematodes



Cadavers are put in white traps

Entomopathogenic nematodes Characterization

- Morphological characterization
- Molecular characterization
- Evaluation of efficacy in the field by Bioassays



Stored in climatic chamber

Juveniles emerging from the *Galleria* larvae are collected and stored in distilled water in 50 ml tubes at 12°C .

Entomopathogenic nematodes

Species	COD	Cultivation	Country of origin
<i>Steinernema carpocapse</i>	ItS-CAO1 ItS-OLE1	Set-aside Tarasco's collection	Italy Italy
<i>Steinernema feltiae</i>	ItS-OT15 It-SOT19 ItS-CO1	Tarasco's collection Tarasco's collection Tarasco's collection	Italy Italy Italy
<i>Steinernema affine</i>	ItS-FO1	Tarasco's collection	Italy
<i>Steinernema arenarium</i>	ItS-OT20	Tarasco's collection	Italy
<i>Steinernema apuliae</i>	ItS-CS1	Tarasco's collection	Italy
<i>Heterorhabditis bacteriophora</i>	IH-LU1	Tarasco's collection	Italy

Moreover, new strains not yet characterized (about 50) have been found and will implement the collection.



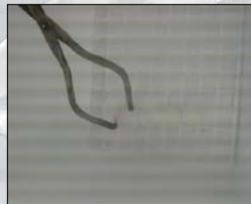
Incubation - 12.000 IJs ml⁻¹ suspended in cryoprotectant for 24-48 h at 24-27°C



Filtration by Whatman filter No. 1 through a vacuum filtration system



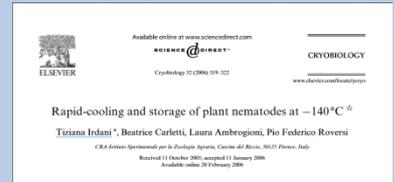
Nylon membranes (Whatman™) roll and place in cryogenic vials



Immediately plunge into LN at -196°C for several minutes

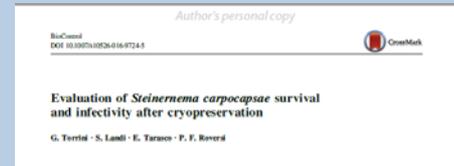
Plant parasitic nematodes

Nematodes are incubated in the 10% (v/v) solution of ethylene glycol for 2 h at 27°C, and subsequently in cold 25% EG for 1 h at 0°C



Entomopathogenic nematodes

- 1) Storage of IJs in distilled water for 15 days at 12°C after their emergence from *G. mellonella* larvae
- 2) 48 h incubation in 18% glycerol at 24°C
- 3) 10 min in 70% methanol at 0°C, several minutes in LN and finally storage in a mechanical freezer at -140°C



6) Nematodes transferred to a mechanical freezer at -140°C



Thawing by incubation in plastic tubes containing Ringer's solution at 24 °C for 4 hours.

Nematode collection
cryopreserved by
liquid nitrogen in
dewar-RIVOIRA

Species	COD	Country of origin
<i>Bursaphelenchus eremus</i>	IT18 IT19 IT21 DE39	Italy Italy Italy Germany
<i>Bursaphelenchus fraudulentus</i>	IT34 IT43 IT23	Italy Italy Italy
<i>Bursaphelenchus hellenicus</i>	IT39 IT41	Italy Italy
<i>Bursaphelenchus minutus</i>	IT42	Italy
<i>Bursaphelenchus mucronatus</i>	IT12 IT4-8 IT13 YACH F2	Italy Italy Italy France
<i>Bursaphelenchus sexdentati</i>	IT40 IT2 IT9 GR-IT	Italy Italy Italy
<i>Bursaphelenchus thailandae</i>	China-A	China
<i>Bursaphelenchus xylophilus</i>	US11 J3 Canada	USA Canada
<i>Heterodera schachtii</i>	IT26	Italy
<i>Globodera tabacum tabacum</i>	IT33	Italy
<i>Meloidogyne incognita</i>	IT30 MA1	Italy
<i>Meloidogyne arenaria</i>	ITPG	Italy
<i>Steinernema carpocapsae</i>	ITS-MR7 CAO1	Italy Italy