Biosystematics of entomopathogenic nematodes: current status and future directions

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ystematics

"… not only classifies and names diversity of the organic world, but seeks to understand the processes that produce and sustain that diversity."

Quicke, 1983

Hmm…
What kind of
nematode
is this?

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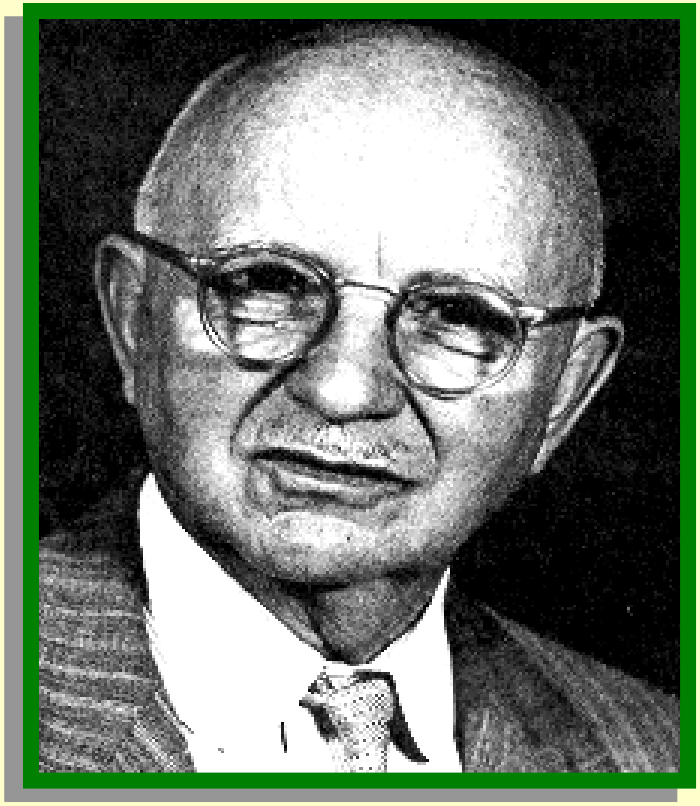
Gotthold von Steiner



1886-1961

Family Steinernematidae Chitwood & Chitwood, 1937

pe genus: Steinernema Travassos, 1927

1. S. kraussei (Steiner, 1923) Travassos, 1927
2. S. glaseri (Steiner, 1929) Wouts, Mracek, Gerdin & Bedding, 1988
3. S. feltiae (Filipjev, 1934) Wouts, Mracek, Boemare, 1994
4. S. affine (Bovien 1937) Wouts, Mracek, Gerdin & Bedding, 1982
5. S. carpocapsae (Weiser, 1955) Wouts, Mracek, Gerdin & Bedding, 1982
6. S. arenarium (Artyukhovsky, 1967) Wouts, Mracek, Gerdin & Bedding, 1982
7. S. intermedium (Poinar, 1985) Mamiya, 1988
8. S. rarum (de Doucet, 1986) Mamiya, 1988
9. S. kushidai Mamiya,1988
10. S. ritteri de Doucet & Doucet, 1990
11. S. scapterisci Nguyen & Smart, 1990
12. S. caudatum Xu, Wang & Li, 1991
13. S. longicaudum Shen & Wang, 1992
14. S. neocurtillae Nguyen & Smart, 1992
15. S. riobrave Cabanillas, Poinar & Raulston, 1994
16. S. cubanum Mracek, Hernandez & Boemare, 1994
17. S. puertoricense Roman & Figueroa, 1994
18. S. bicornutum Tallosi, Peters & Ehlers, 1995
19. S. monticolum Stock, Choo & Kaya, 1996
20. S. oregonense Liu & Berry, 1996
21. S. abbasi Elawad, Ahmad & Reid 1997
22. S. ceratophorum Heng, Reid & Hunt, 1997
23. S. karii Waturu, Hunt & Reid, 1997
24. S. siamkayai Stock, Sommsook & Reid, 1998
25. S. tami Van Luc, Nguyen, Reid, Spiridonov, 2000
26. S. thermophilum Ganguly & Singh, 2000
27. S. sangi Ke, Nguyen & Moens, 2001
28. S. loci Ke, Nguyen & Moens, 2001
29. S. thanhi Ke, Nguyen & Moens, 2001
30. S. pakistanense Shahina, Anis, Reid, Rowe and Maqbool, 2001

Neosteinernema Nguyen & Smart, 1994

1. N. longicurvicauda Nguyen & Smart, 1994

Family Heterorhabditidae Poinar, 1976

Type Genus: Heterorhabditis Poinar, 1976

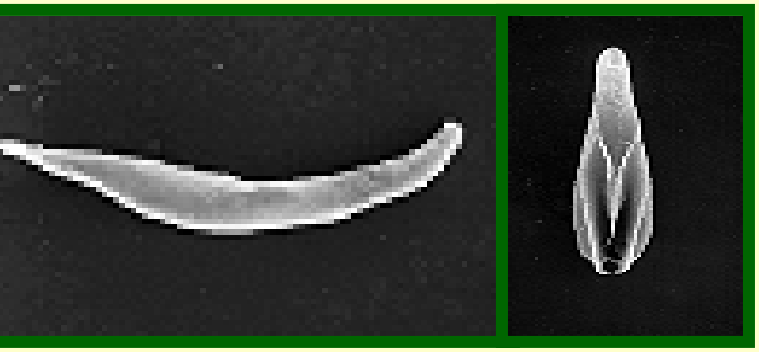
1. Heterorhabditis bacteriophora Poinar, 1976
   syn. Chromonema heliothidis Khan, Brooks & Hirschmann, 1976
   Heterorhabditis heliothidis (Khan, Brooks & Hirschmann, 1976) Poinar, Hess & Thomas, 1977
2. H. megidis Poinar, Jackson & Klein, 1988
3. H. zealandica Poinar, 1990
4. H. indica Poinar, Karunakar & David, 1992
   syn. H. hawaiiensis Gardner, Stock & Kaya, 1994
5. H. argentinensis Stock, 1993
6. H. brevicaudis Liu, 1994
7. H. marelatus Liu & Berry, 1996
   syn. H. hepialius Stock, Strong & Gardner, 1996
8. H. poinari Kakulia & Mikaia, 1997
9. H. downesi Stock, Griffin and Burnell, 2002

Description Criteria in Currently Described EPN Species

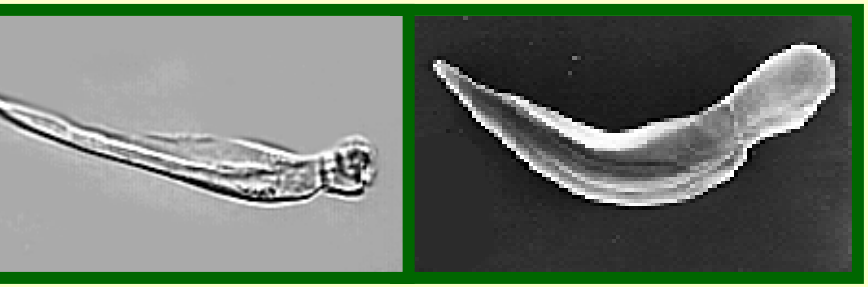
- Phenetic species concept → Morphology
- Biological species concept → Cross-breeding tests

Key Diagnostic Features

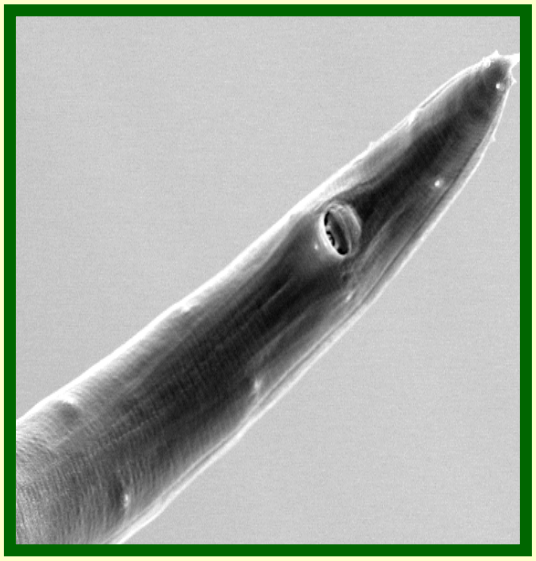
st. Generation Male



Gubernaculum



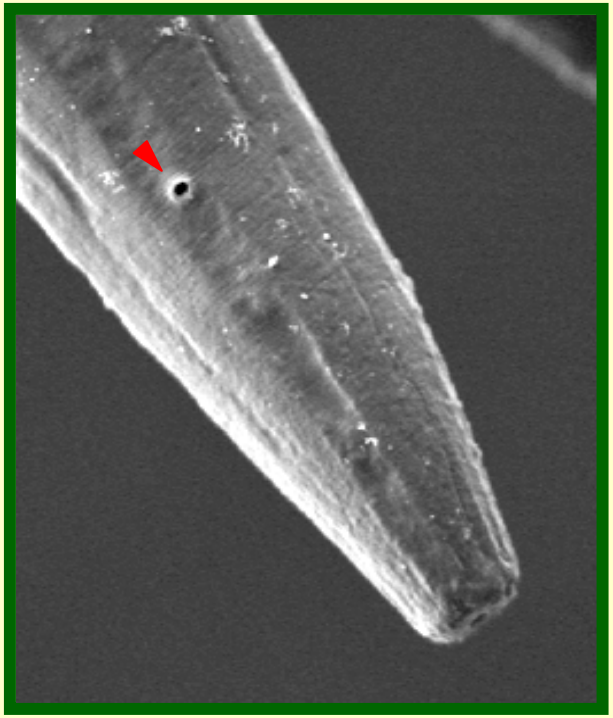
Spicules



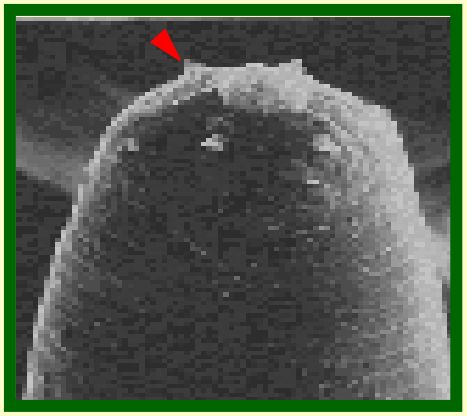
Tail,
Genital papil

Key Diagnostic Features

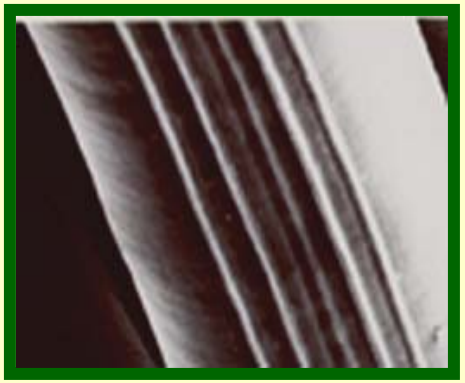
rd Stage Infective Juvenile:



Excretory pore location



Cephalic oddities



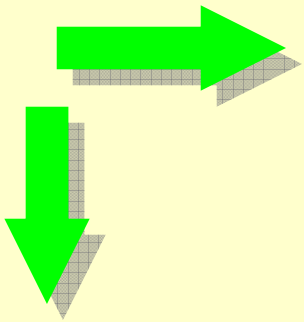
Lateral field pattern

Limitations of currently used methods

Lack of morphological variation at the specific level.

Most morphological diagnostic traits have no evolutionary ue because they represent either plesiomorphic states or are hly homoplasious characters.

Cross- Hybridization Methods



- ✓ Time consuming
- ✓ Too laborious

A self-fertile species of *Steinernema* from Indonesia: further evidence of convergent evolution amongst entomopathogenic nematodes?

C. T. Griffin, K. M. O'Callaghan and I. Dix. Parasitology (2001) 122, 181-186

Molecular Approaches

- **Protein electrophoresis:** Isozyme patterns — 1960's
  Total protein patterns — 1980's
- **Immunological techniques:** Gel diffusion, Immunoelectrophoresis — 1980's, 1990's
- **RFLPs** — 1980's - present
- **RAPDs** — 1990's -present
- **Nucleotide sequence analysis** — late 1980's - present

Assessment of Molecular Approaches for EPN Diagnostics

Protein electrophoresis

- Good at species level **(sibling species)**
- Little use above the genus level, or below the level of subspecies
  (unless levels of divergence are present)

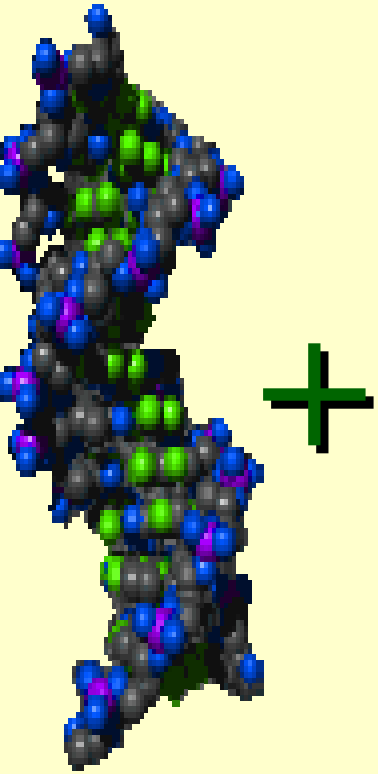
Immunological techniques

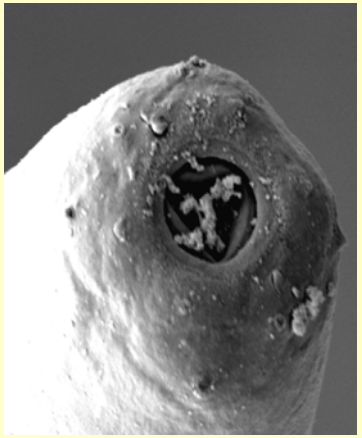
- Useful at the species level

Assessment of Molecular Approaches for EPN Diagnostics (cont.)

- **RAPDs**
  - Useful at the subspecies level (populations, strains)
- **RFLPs**
  - Useful at species levels and infra-specific categories
- **Nucleotide sequence analysis**
  - Depending on the gene and gene-region target this method can be valuable for discriminatin taxa above, below or at the species level

combined morphological-molecular approach.





A more comprehensive view of EPN evolution

A better classification

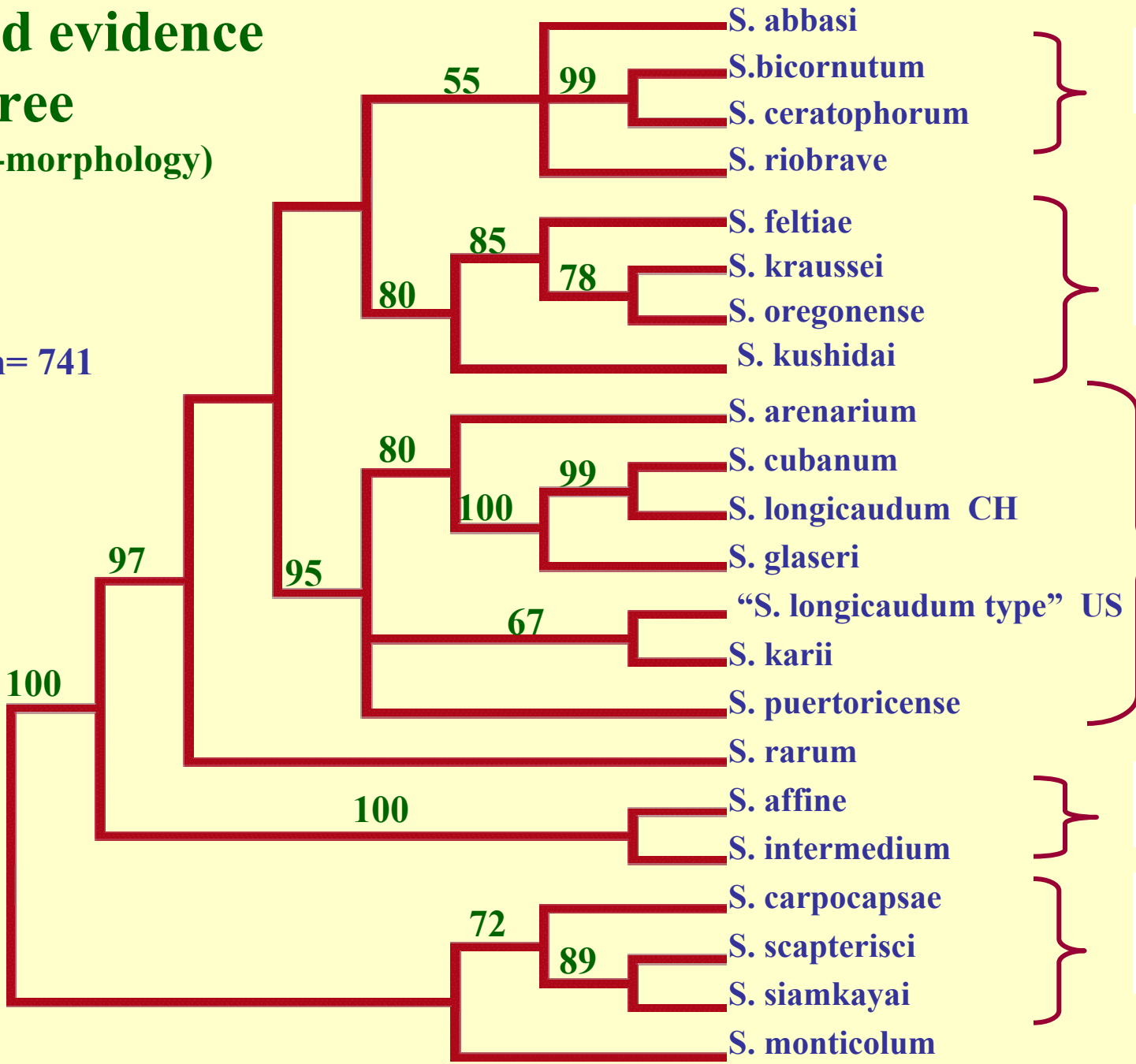
hylogeny of *Steinernema* Travassos, 1927 (Cephalobina: Steinernematidae) nferred from ribosomal DNA sequences and morphological characters.
tock, Campbell and Nadler, 2001. J. Parasitol. 87: 877-889.

- **Evolutionary relationships among *Steinernema* spp.**
- **Patterns of character evolution.**
- **Apply the "autapomorphy-base concept for delimitation of *Steinernema* species.**

mbined evidence tree

molecules-morphology)

ree length= 741
.I.= 0.60



IJs with horn-like structu

Medium size IJ
Average:
650-900 µm

Large IJ
Average
More than 90

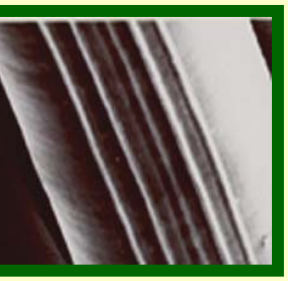
Similar adult an
IJ morphology

Small IJs
Average:
Less than 600µ

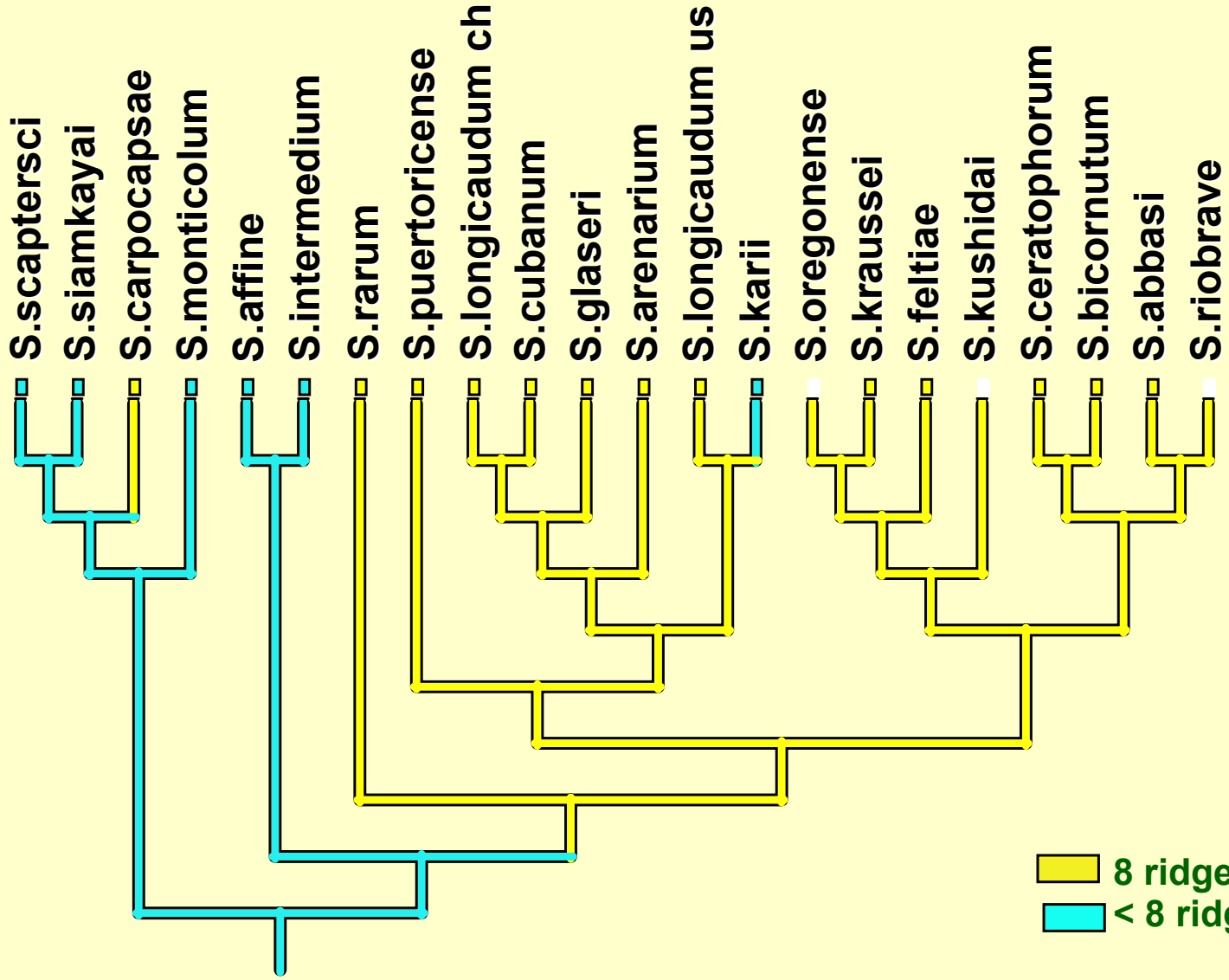
Most of the morphological characters studied were...

Homoplasious → **non-homologous, variable results according to different tree-topologies**

Plesiomorphic → **ancestral states**



IJ lateral field pattern



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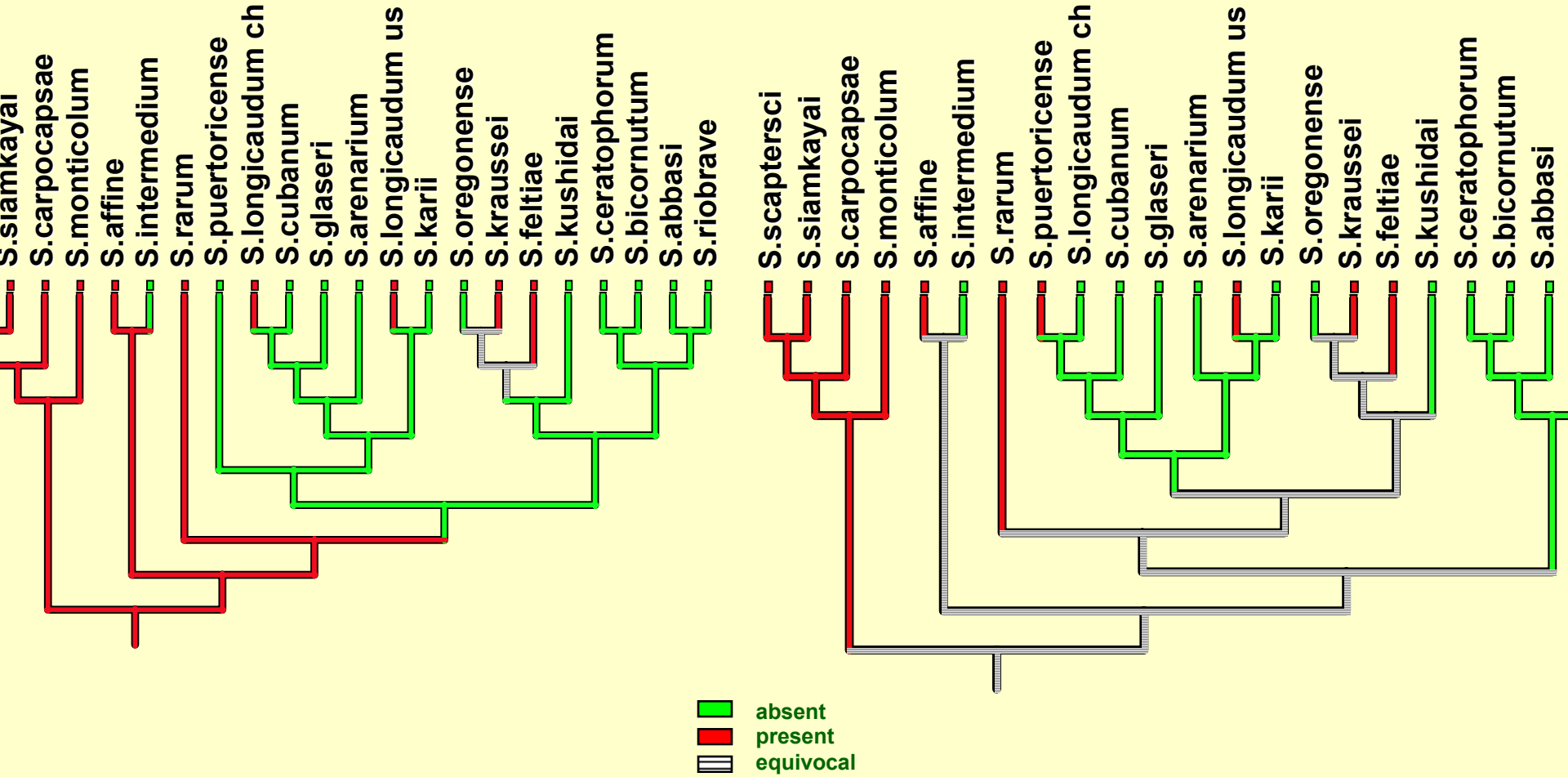
Male:tail mucro



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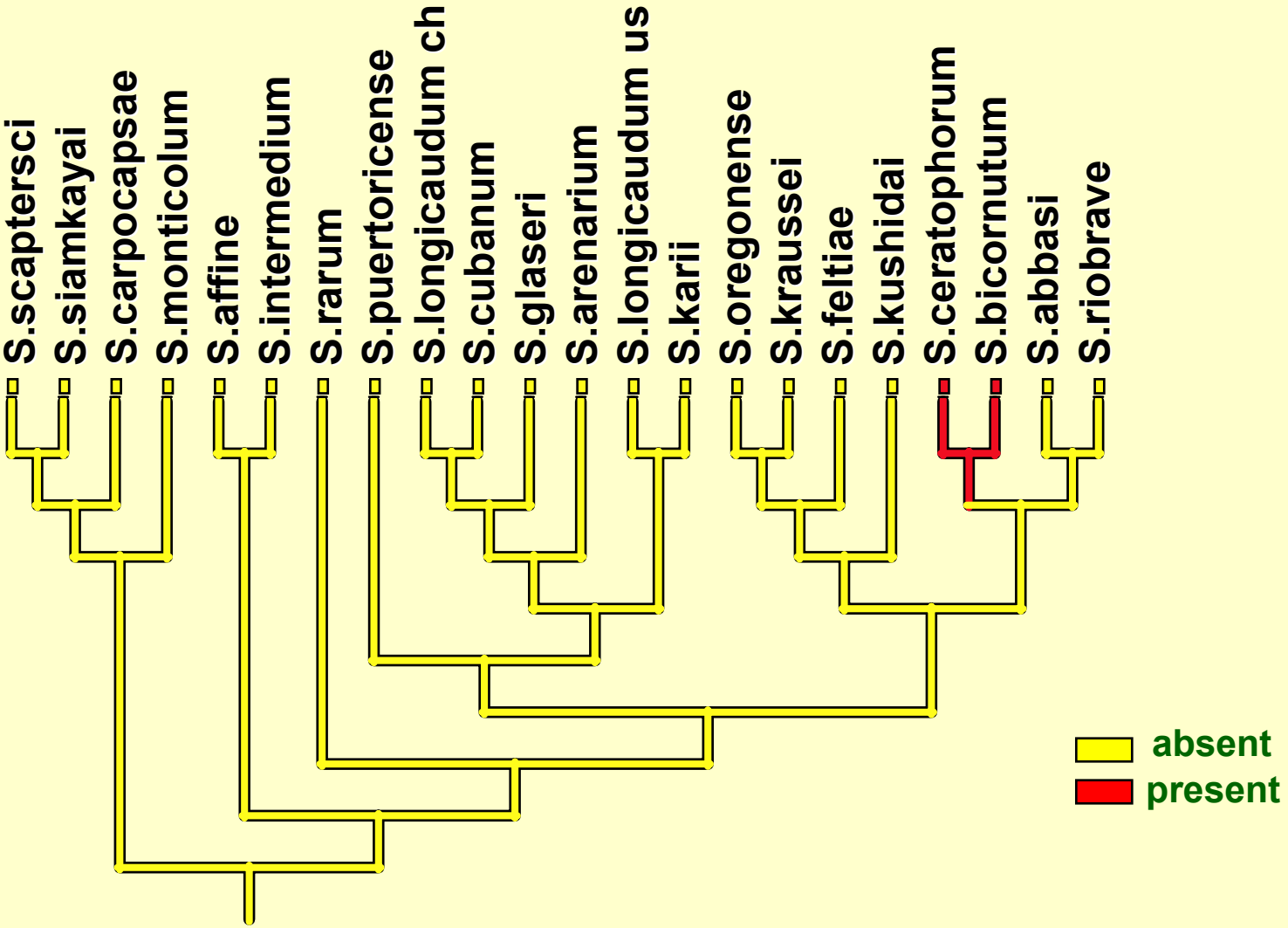
IJ: Horn-like structures



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Species Delimitation → "Automoporphy Concept

- 4 combined evidence trees (morphology + LSU) → All taxa delimited by 3 or more autapomorphies

- 2 LSU trees (full alignment dataset) → All taxa delimited by 2 or more autapomorphies except *S. carpocapsae*

ummarizing…

This study provides a unifying framework where molecular and morphologica
ols have demonstrated to be complementary tools in EPN systematics.

This combined morphological-molecular approach has shown to be
uable not only for understanding evolutionary relationships among species
genus *Steinernema,* but also for interpreting patterns of character evolution
rphological traits and in assessing diagnosable units in this genus.

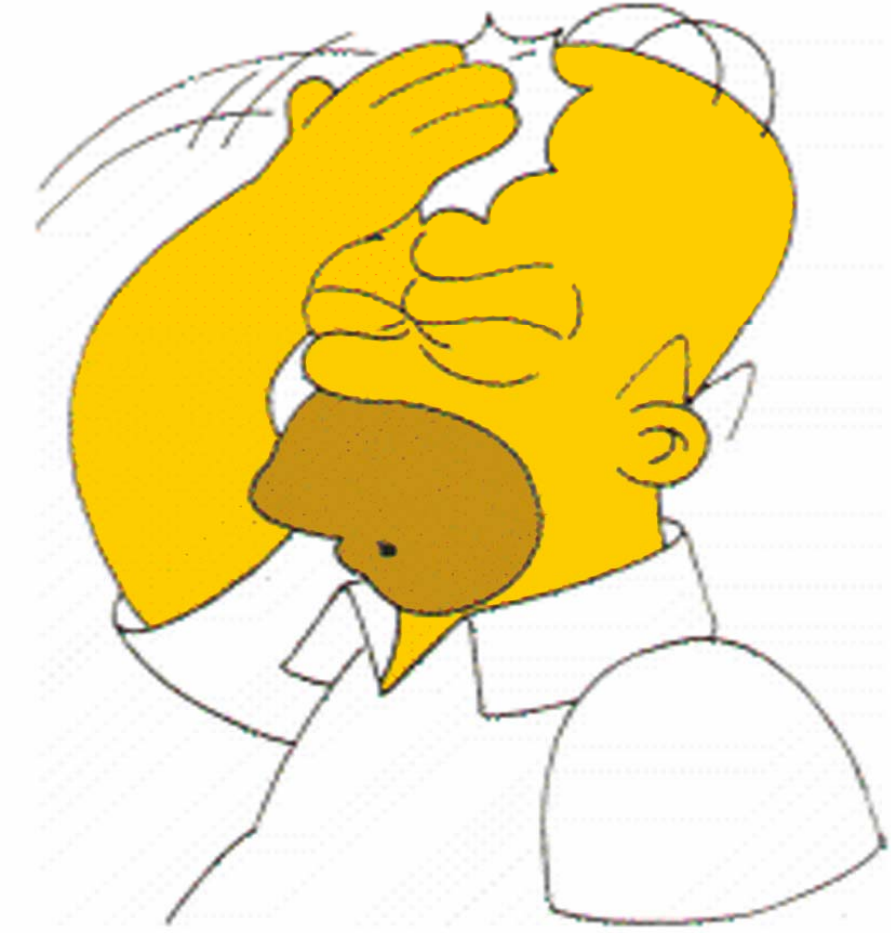
Introductory slide to Alex’s presentation

Single site sampling strategy

- 100 soil samples taken from a 100m^2 grid.
- Each sample baited with 3 *Galleria* larvae.
- Progeny from each larvae analyzed separately.
- Soil samples baited at several time points.

- Should give an indication of genetic diversity over space and time.

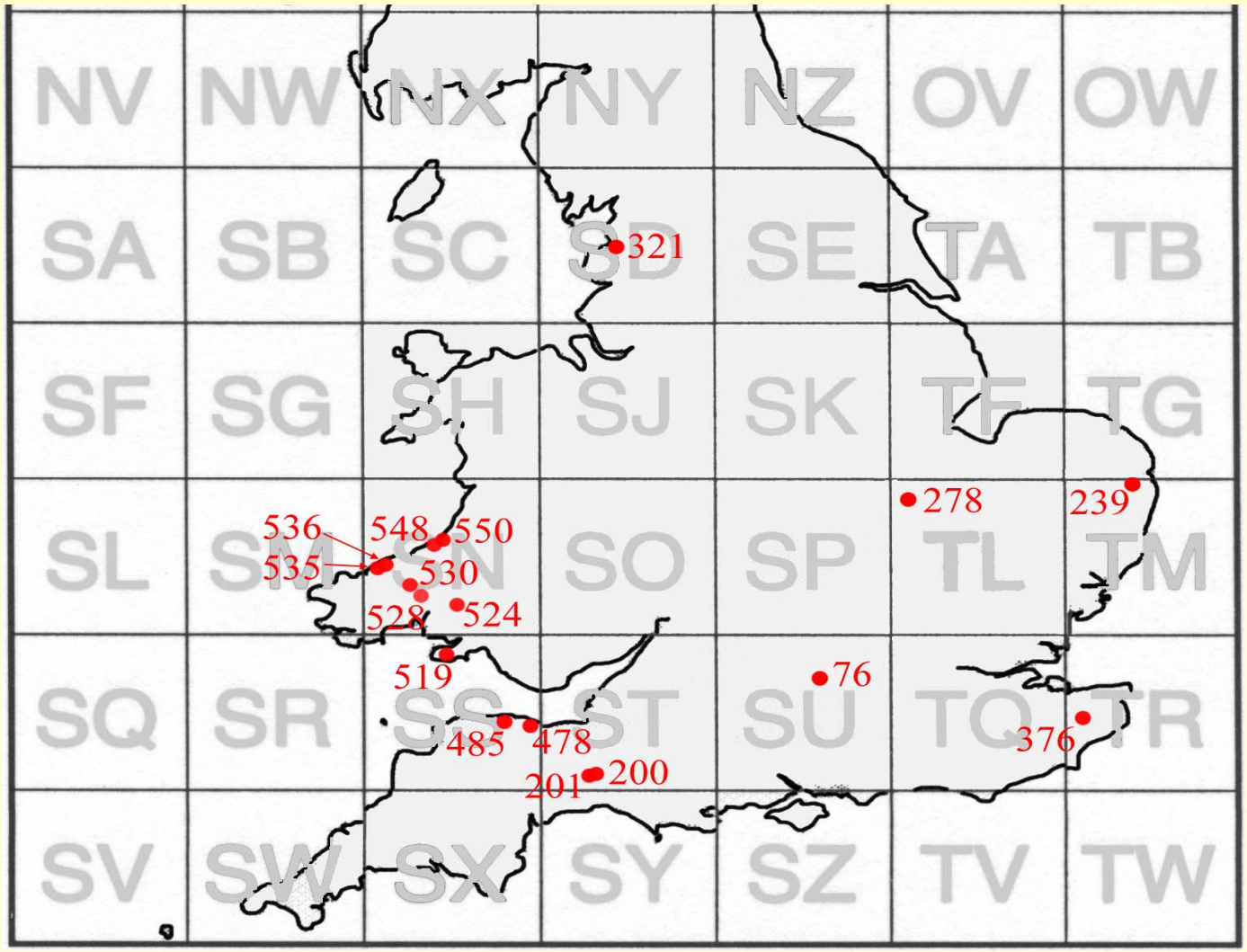
D'OH



S. feltiae sites

Site number	Grid reference	Habitat type
76	SU 568 729	Hedgerow
200	ST 280 108	Field - Pasture
201	ST 277 088	Field - Pasture
239	TM 396 995	Field - Cultivated
278	TL 096 874	Wood - Deciduous
321	SD 414 453	Field - Cultivated
376	TR 114 484	Field - Cultivated
478	SS 936 458	Verge
485	SS 802 483	Moorland
519	SS 412 812	Field - Pasture
524	SN 387 213	Field - Pasture
528	SN 288 277	Field - Pasture
530	SN 221 343	Field - Pasture
535	SN 066 406	Verge
536	SN 072 424	Field - Pasture
548	SN 345 555	Verge
550	SN 366 572	Field - Pasture

UK *S. feltiae* site locations



Three fingerprinting techniques used

AFLP

RAPD

ISSR

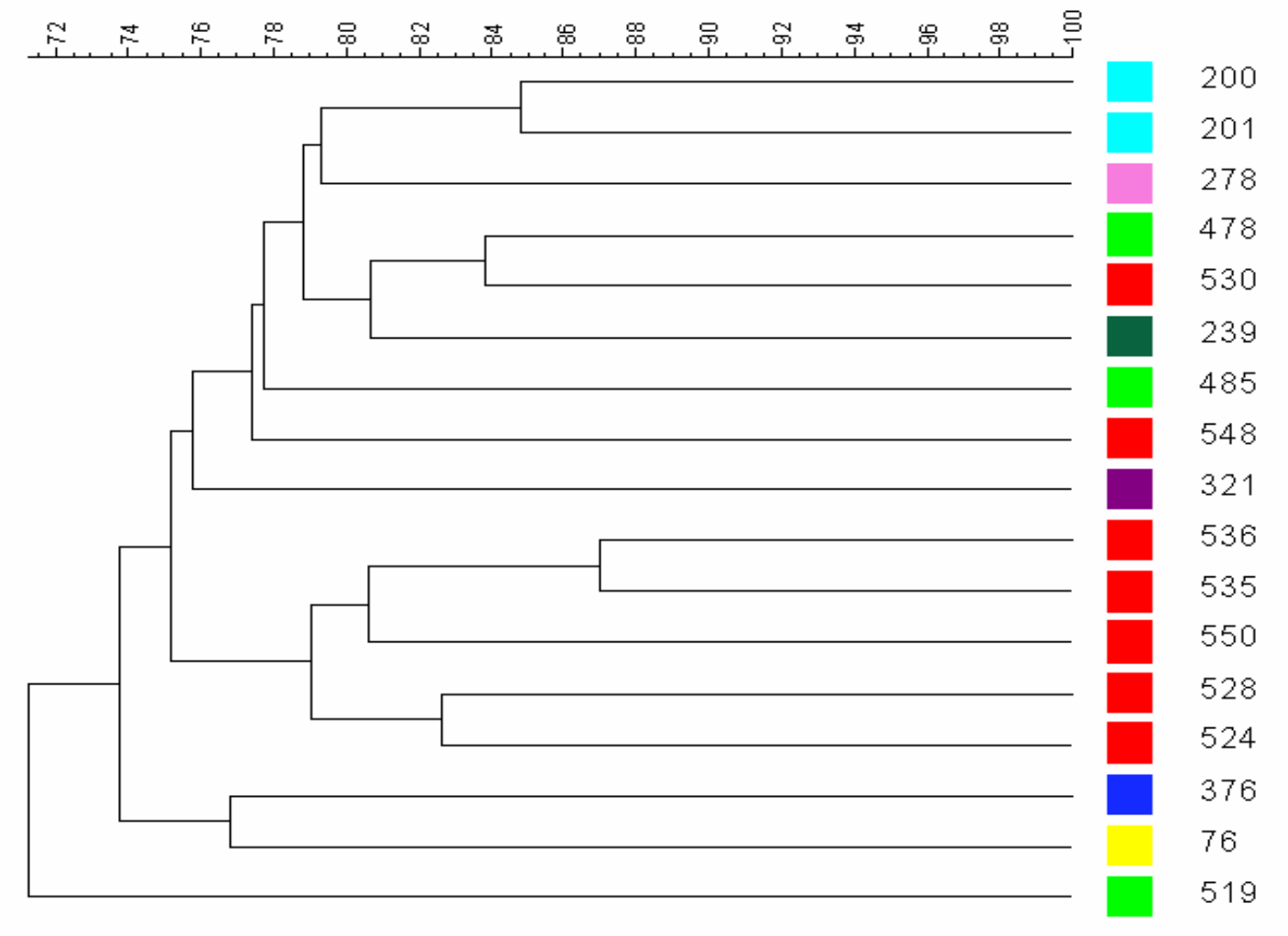
ISSR technique

Interspersed Simple Sequence Repeats

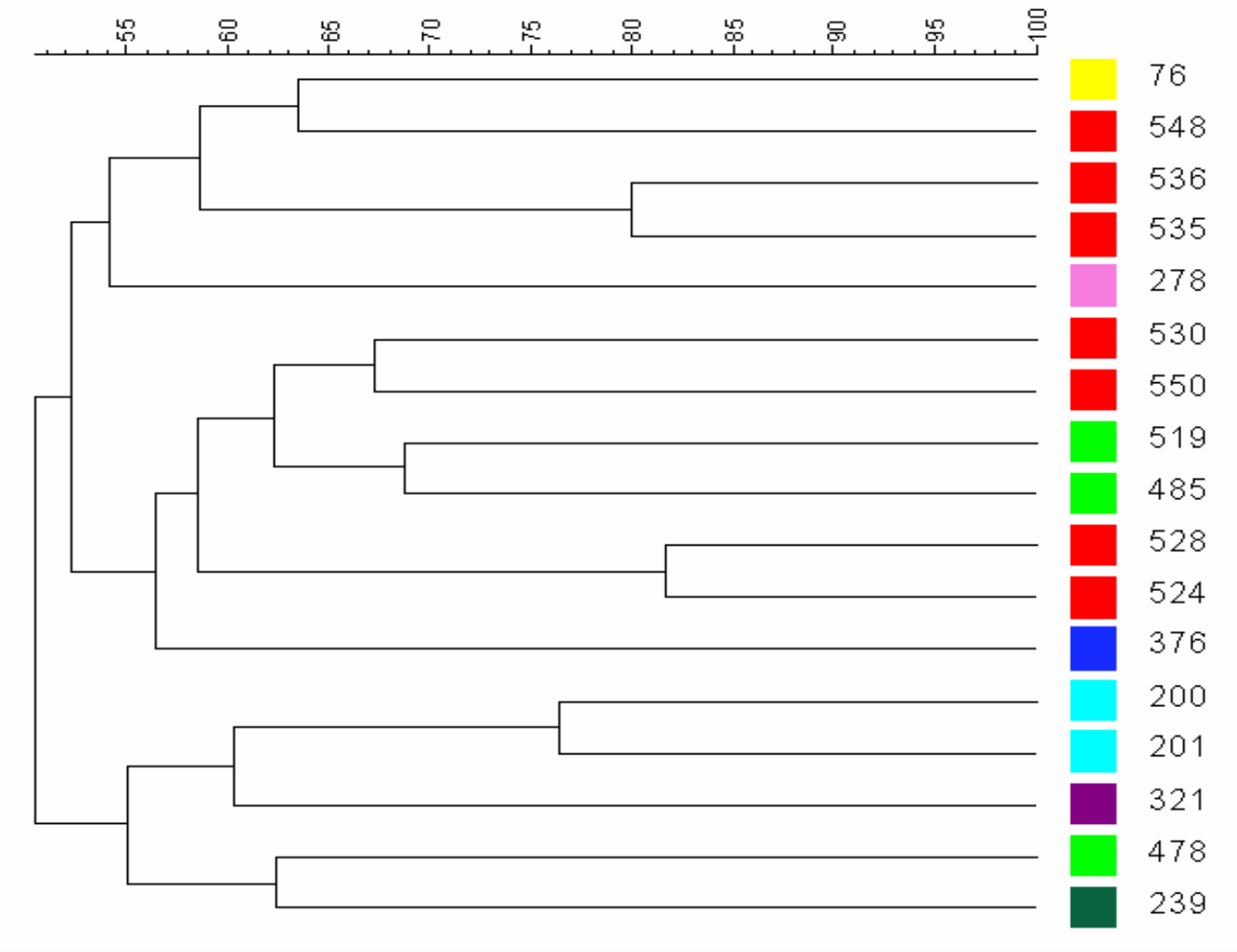
- Primers have a core microsatellite sequence with a degenerate 5’ end

  BDB $(ACA)_5$ or DD $(CCA)_5$
- Amplify regions of genomic DNA flanked by microsatellite like sequences.
- Random distribution of microsatellite sequences in the genome yield polymorphic bands.

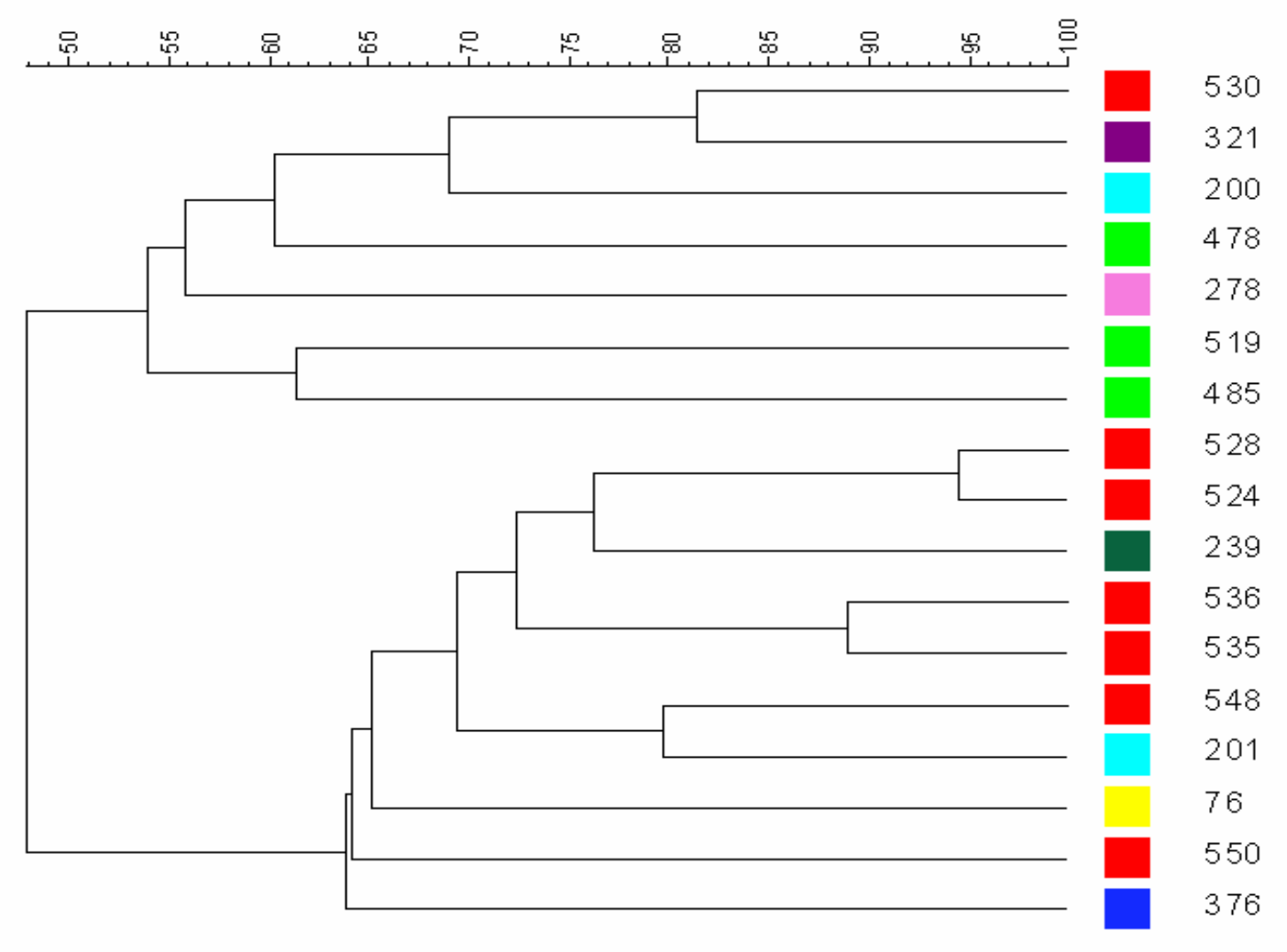
S. feltiae (AFLP data)



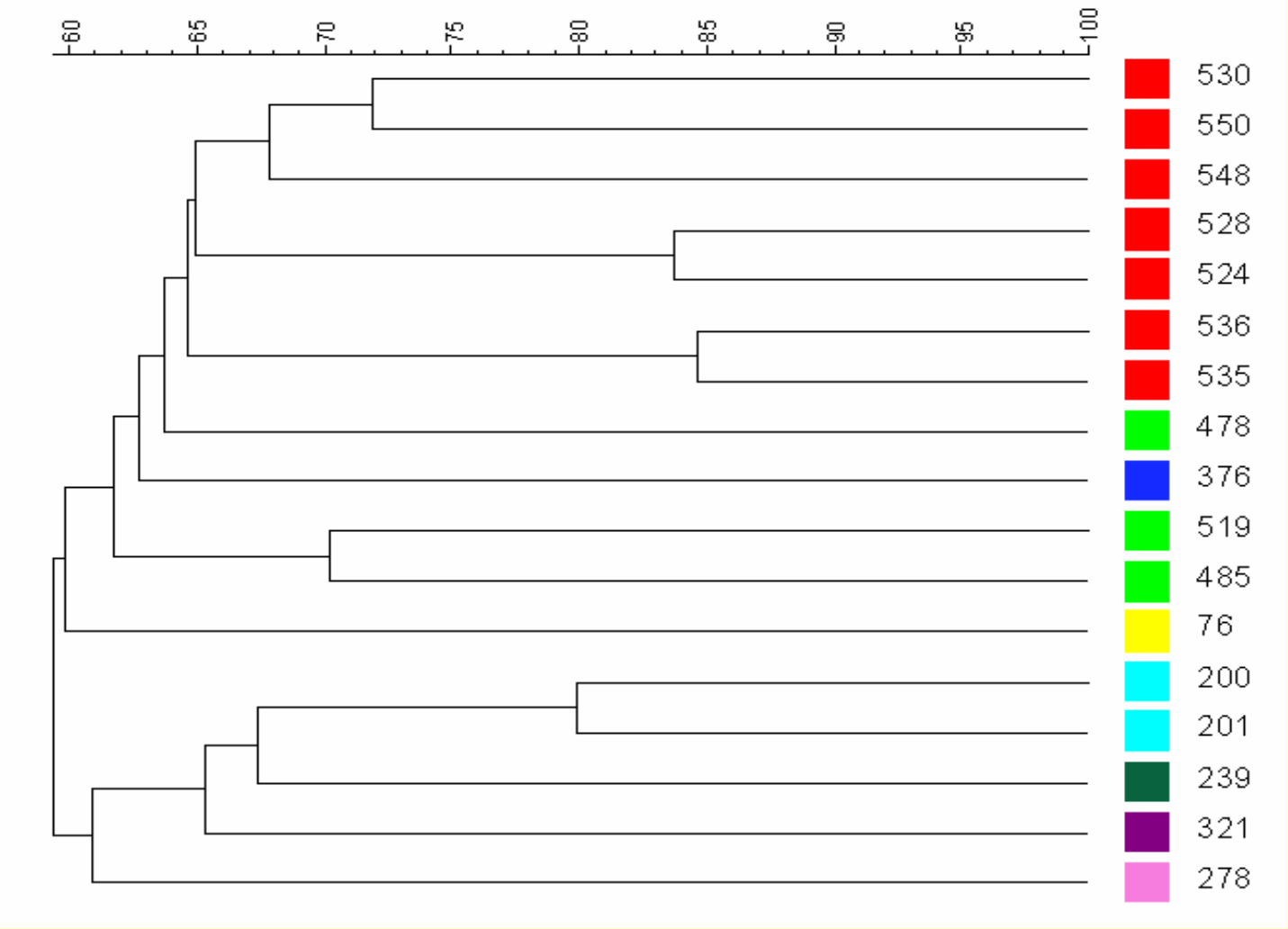
S. feltiae (RAPD data)



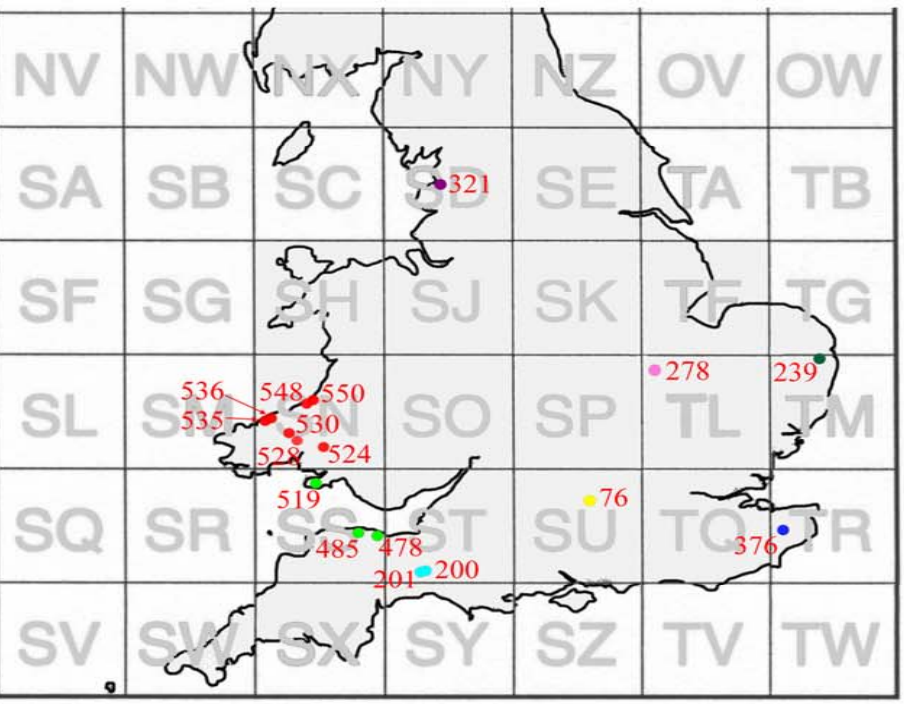
S. feltiae (ISSR data)

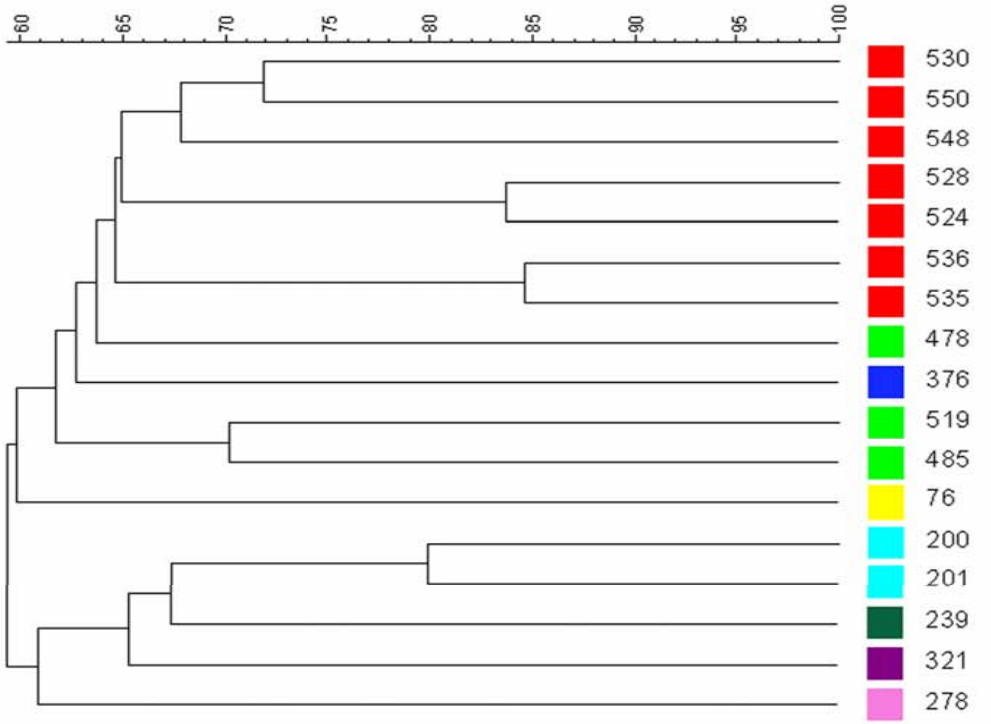


S. feltiae (combined data)



S. feltiae (combined data)





Conclusions - UK *S. feltiae* samples

- Three different fingerprinting approaches gave different results.
- No good correlation between geographical location and diversity for individual techniques.
- Correlation between geographical location and genetic diversity. (when data for all three methods combined).