High Throughput Sequencing of Nematode Communities

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Introduction

Nematodes are an important component of the soil food web.

Nematode community composition may be used in assessing soil health[1].

Morphological identification of nematodes is time consuming and specialised. Juvenile nematodes can be problematic and there are cryptic species.

Molecular methods may offer an alternative for ecological studies[2] with advantages in time, objectivity and level of training required.

We aim to develop a high throughput screening method for nematodes.

Methods

PCR amplification of near full length 18s rDNA

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Cloning

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Sequencing reaction performed with 3 primers

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Full length single strand constructed

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Full length sequences aligned in POA

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Neighbor joining tree drawn (F84 with gamma rates)

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35 types identified (clustered at 0.01 substitutions per base)

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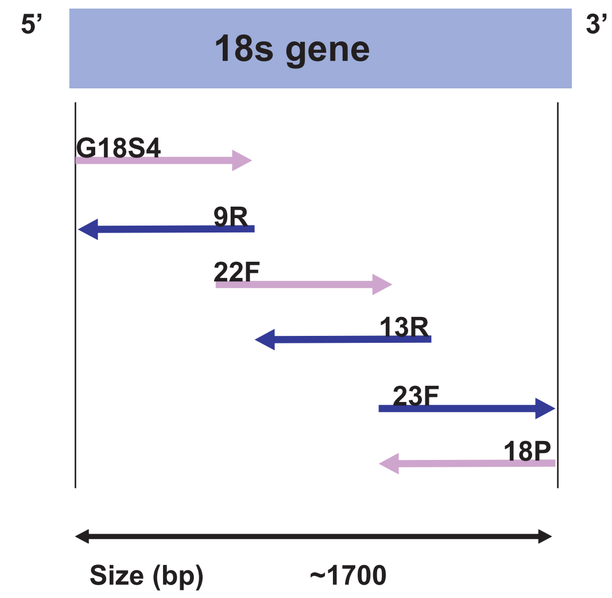
Up to 4 clones from each type were selected for double stranded sequencing

↓

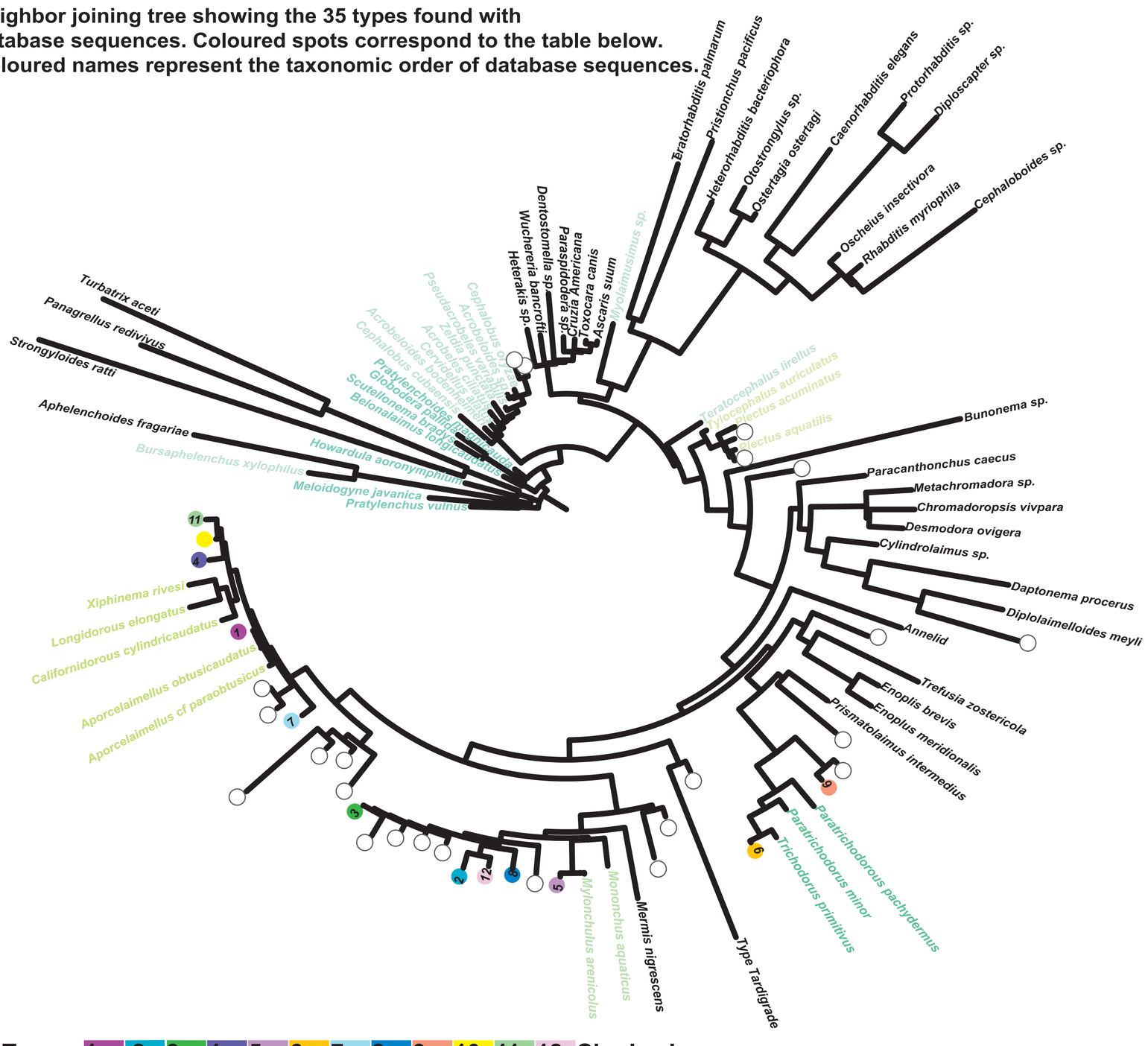
Sequences aligned with database sequences and tree redrawn



Primers used for sequencing 18s ribosomal DNA. First strand sequencing was performed with primers indicated in purple. Double strand sequencing was completed with primers in blue.

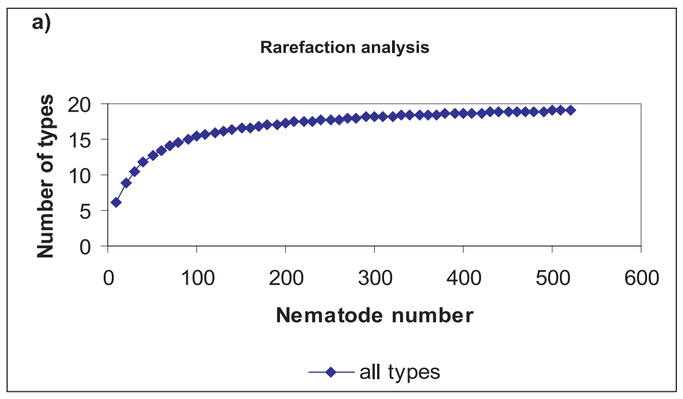
Results

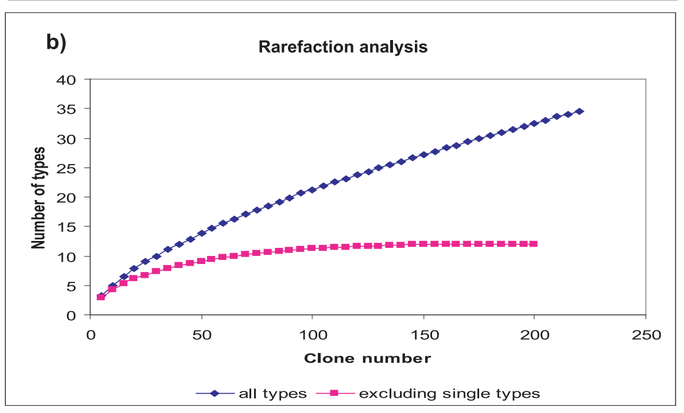
Neighbor joining tree showing the 35 types found with database sequences. Coloured spots correspond to the table below. Coloured names represent the taxonomic order of database sequences.



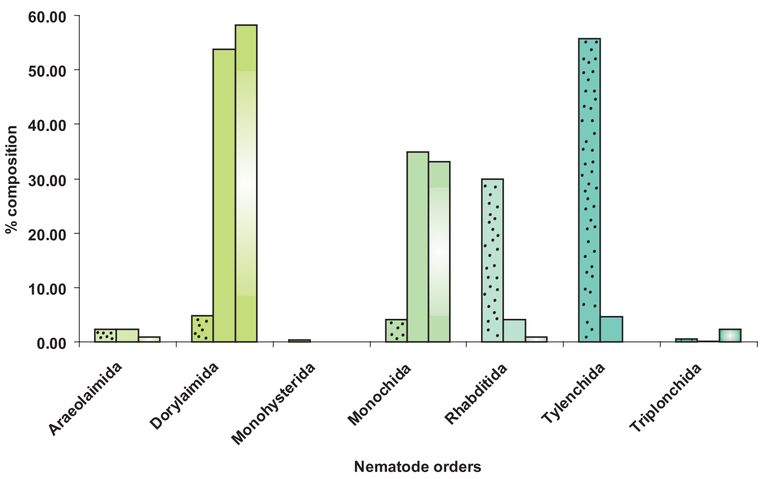
Type	1	2	3	4	5	6	7	8	9	10	11	12	Single clones
Plot 1	46	9	12	4	4	1	0	4	3	1	2	2	14
Plot 2	63	23	10	3	2	4	5	0	0	2	1	0	9
Total	105	32	22	7	6	5	5	4	3	3	3	2	23

Number of clones of each type found in samples from each of the plots. Colours correspond to the above tree.





Rarefaction analyses show a) morphological types were adequately sampled and b) the clone library was adequately sampled, except for single clones each of which make up <0.5% of the population.



% composition of the community as calculated from number of indiviuals , biovolume and number of clones . Colours correspond to the names on the tree.

Conclusions and Future Study

Full length 18s ribosomal DNA sequence can be used to distinguish nematode types (genus level).

Rarefaction analysis suggests that both morphological and molecular analysis, excluding single clone groups, was sufficient.

The main disparity between molecular and morphological findings may be explained by biovolume.

Problems with amplifying Rhabditids and Tylenchids are currently being addressed using new primers and separation of large and small worms during extraction.

The sequences obtained here may be used to develop T-RFLP strategies allowing rapid study of nematode communities[3].

References

[1]Bongers,T. (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19

[2]Floyd,R., Abebe,E., Papert,A., Blaxter,M. (2002) Molecular barcodes for soil nematode identification. Molecular Ecology 11:839-850

[3]Griffiths,B.S., Donn,S., Neilson,R., Daniell,T.J. (2005) Molecular sequencing and morphological analysis of a nematode community. Applied Soil Ecology (in press, available online)

Acknowledgements

This work was performed as part of my Masters (University of Dundee & University of St Andrews). The project is continued under a BBSRC Quota Studentship.