SOIL Discuss., 2, C679–C684, 2016
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SOIL Discussions Open Access EGU

Interactive comment on "Nematode taxonomy: from morphology to metabarcoding" *by* M. Ahmed et al.

Anonymous Referee #2

Received and published: 14 January 2016

Nematode taxonomy: from morphology to metabarcoding

In this work the history and progress in nematode systematics is discussed, subsequently an overview of molecular methods for nematode identification is given. In the last two decades the use of molecular techniques for nematode identification and quantification has increased enormously, and the implications of this development both for nematode taxonomy, ecology and practical applications should be discussed. However, the manuscript in its current form try to covers too many aspects / field, lacks novelty and the main message is unclear. Therefore it requires a very thorough revision, and the removal of multiple sections (see below) to be suitable for publication in the SOIL journal. This is illustrated by some specific comments below.

Abstract: "Some groups of nematodes are also known to cause significant losses to

crop production" – apparently the authors refer to plant-parasitic nematodes

". . . knowledge of their diversity is still limited due to the difficulty in achieving species identification using morphological characters" Virtually all (if not all) species known so far are defined on morphological and/or histological autapomorphies. Hence, we can't determine whether our "knowledge of their diversity is still limited" due the abovementioned difficulty. I can relate 'diversity' to "species definition" or equivalent, but not to species identification. In short: a hard-to-understand statement

". . . useful means of circumventing the numerous limitations associated with classical morphology based identification" No, it is circumventing anything – it is just (enormously) the number of informative characters. There is no fundamental difference between morphological or DNA sequence-based characters.

"the limitations of classical taxonomy" – avoid the term "classical". It is just about the number of characters, whether they are dependent or not, whether they can be easily scored, and whether their polarity can be determined.

"high throughput sequencing is facilitating advanced ecological and molecular studies". Rather, HGT allows for a shift in terms of time (and – therefore – resources) from data collection to data analysis. It gives researchers the opportunity to analyze numbers of samples (and sample size) that are required for proper statistical analyses (and not dictated by "what can maximally be handled by a limited number of people"). Whether an ecological study is 'advanced' depends on other things.

Introduction: "the criteria for allocating individuals into these groupings have often been questioned since even species within the same trophic group are known to sometimes vary in their source of food and response to disturbances" More fundamental point of criticism – the usefulness/validity of 'trophic groups' depends very much on the underlying research question. If this question is about carbon or nitrogen fluxes through a soil food web, this might be valid. For more detailed questions, it should be noticed that "trophic groups" are composed of phylogenetically fully unrelated taxa that only have

SOIL
2, C679–C684, 2016

one thing in common – they roughly (!) prefer the same kind of food.

“for species level identification is vital to accurate and precise computation of nematode indices as determiners of sediment quality” – At species level? For by far most free-living nematodes virtually no ecological information is available at species level. Hence, there is no reason to label this as being ‘vital’.

“as well as the existence of intraspecific variations and cryptic species (valid species species that morphologically indistinguishable)” – for the purpose indicated here (“computation of nematode indices as determiners of sediment quality” (what about soil?)), I would suggest not to put any effort in such subtleties (there are many, more basic hurdles to be overcome). Note “species species”.

“categorizing nematodes based on higher level classifications such as families and feeding guilds” – again, the taxonomic resolution required is variable will be defined by the underlying research question.

“. . . recently made some very important modifications to its policy” “(Regulation 2009/1107/EC OL and Directive 2009/128/EC)” – Recently? This is 7 years ago.

“These alternative approaches will undoubtedly rely” – why the two most important ones, crop rotation and host plant resistances, are not mentioned?

“the differential host test (Sasser, 1954), scanning electron microscopy (Eisenback and Hirschmann, 1981; Charchar and Eisenback, 2000; Eisenback and Hunt, 2009), biochemical approaches such as isozyme electrophoresis” These techniques are used for very distinct (and non-comparable) reasons: host tests for pathotyping, SEM for the generation of additional morphological characters, and isozyme analysis for species identification (actually also life stage identification).

“molecular methods of plant parasitic nematode identification discussing in depth the different markers and DNA target regions used for discriminating species, their future prospects and limitations (Powers et al., 1997; Powers, 2004; Blok, 2004, 2005)”. (. . .

SOIL
2, C679–C684, 2016

I'm afraid with quite some overlap wit the current MS)

The phylum Nematoda: "as Priapulida, the Kinorhyncha as well as their closest sister taxon the Nematomorpha". Non-relevant in this context, covered in more detail in various other recent papers.

"This has however been disputed by De Ley (2000) and De Ley et al. (2005) who argued that this theory simply emanates from the failure of light microscopy to provide enough resolution, thus precluding" – skip, in 2016 this is a non-discussion / irrelevant.

"which is a relatively small fraction of the predicted number of species of ca. 1 million (Hugot et al., 2001)" – speculation about number of extant nematode species should be discussed in full detail or left out. In the context of this MS, I tend to opt for the latter.

"To properly deal with the issue of, De Ley (2000) suggested that reassessment of priorities is the best way to progress. He cited a number of steps to achieve this: . . ." Skip, irrelevant for this MS

Predicted species diversity leaves so much more to do / Classical taxonomy and the vast taxonomic deficit Skip whole sections: speculation of number of species is not useful. Complaining in the same section about the decline of the number of taxonomists is quite "preaching to your own choir"-like. This is not the forum to do this.

Changes within the classification systems: Too much overlap with (for instance) Systematic Position and Phylogeny by Paul de Ley and Mark Blaxter (De Ley P, Blaxter ML. 2002. Systematic position and phylogeny. In: Lee DL, editor. The biology of nematodes. London: Taylor & Francis. p 1–30).

6 Biochemical methods for nematode identification Skip all the historical overview-like elements 6.1 Protein based approach. For systematics and identification this outdated (key reason: protein expression depends on life stage / environmental conditions etc. – hence, unstable as marker) 6.2 DNA based approach p. 1189, lines 9-10: "The two ITS regions have been used in the past both as phylogenetic and diagnostic markers".

SOIL

2, C679–C684, 2016

Interactive Comment

Right, ITS regions are very problematic as diagnostic marker. Two quotes from recent articles: “ITS sequences were studied to develop species-specific primers used in simple PCR reactions, e.g. , for detection of H. glycines (Subbotin et al. , 2001) and H. schachtii (Amiri et al. , 2002). However, polymorphism between rDNA repeats within a species like H. latipons makes designing a species-specific primer very difficult (Rivoal et al. , 2003)” (from Toumi et al. in Nematology 15 (2013) 709-717) “Moreover, polymorphism between ribosomal DNA (rDNA) repeats can occur within one species, e.g. H. avenae (Bekal et al. 1997; Zhao et al. 2011) and H. filipjevi (Subbotin et al. 2000; Subbotin et al. 2003). This polymorphism makes the design of a species-specific primer based on ITS-sequences very difficult” (from Toumi et al. in Eur J Plant Pathol (2013) 136:613–624) - suggestion: skip the section on ITS based identification (p. 1189. Line 3 – p. 1190, line 2.

p. 1193 (lines 19-23). “It should, however, not be confused with metagenomics, a term often used to refer to the genomic analysis of organisms from environmental samples (Handelsman, 2004; Tringe et al., 2005; Hugenholtz and Tyson, 2008). Another form of environmental DNA analysis that is just as common as, and often albeit wrongly used as synonym of, metagenomics is metagenetics” None of the authors are authorities in this field – hence skip & refrain from making strong statements on this topic

7 Limitations of high throughput DNA barcoding. p. 1195, lines 5-6. “It has however, been shown to have limited taxonomic resolution among certain taxa within the phylum Nematoda”. Note there is no “one-for-all” – so far SSU rDNA is the only one with reasonable phylum-wide coverage

p. 1195, lines 14-15. “Another issue with DNA metabarcoding is its reliance on PCR (Taberlet et al., 2012). Significant amount of errors have been shown to accrue during amplification”. Worthwhile mentioning: most of the time it is just improper use (!).

8 Next generation sequencing technology p. 1196, lines 16-25. Skip, do the scientific community a favor, and don’t explain Sanger sequencing here (!) –

SOIL

2, C679–C684, 2016

Note that 454 sequencing is almost phased out. In short: skip the historical overviews, and focus on current and near future approaches.

Interactive comment on SOIL Discuss., 2, 1175, 2015.

SOIL

2, C679–C684, 2016