SOIL Discuss., 2, C733–C741, 2016
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Interactive comment on "Nematode taxonomy: from morphology to metabarcoding" *by* M. Ahmed et al.

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Received and published: 10 February 2016

Anonymous reviewer 2,

The authors are very grateful for your helpful comments and suggestions on this manuscript. Please see our responses to your comments and suggestions below .

On the general comments about the main message being unclear, the authors propose to review the introduction to ensure that the aim of the work is clearer.

Reviewer's comment: Abstract: "Some groups of nematodes are also known to cause significant losses to crop production" – apparently the authors refer to plant-parasitic nematodes

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Response: Indeed, the reference is to plant-parasitic nematodes. We propose rephrasing that sentence to: “Some plant-parasitic species are also known to cause significant losses to crop production”

Reviewer’s comment: ” . . . 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 above mentioned difficulty. I can relate ‘diversity’ to “species definition” or equivalent, but not to species identification. In short: a hard-to-understand statement

Response: Yes, it is true that almost all known species are defined on the basis of morphological/phenotypic characters. However, there is evidence of valid species bearing striking morphological resemblances to others such that even some expert taxonomists cannot accurately distinguish them. We also can see how the Reviewer can better relate diversity to species definition rather than species identification, since whatever concept of species ‘we’ decide to adopt can have huge impact on our perceived understanding of diversity. Therefore, in the above quoted line from the manuscript, the appropriate word perhaps could be “delineation” instead of “identification”.

Reviewer’s comment: “... 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.

Response: What is inferred here, as you mentioned, is that DNA provides more informative characters which in the case of morphology can be limited. As a consequence, this means that for some taxa this limited number of informative characters may not be enough to reach species identification. Under such situations, therefore, the DNA approach is helping to overcome this limitation by offering informative characters to

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base species identifications/delineation on. We hereby propose rewriting this sentence as: “Molecular methodology has provided a useful means of overcoming the limited availability of reliable diagnostic characters associated with morphology-based identification”.

Reviewer’s comments: “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.

Response: True, NGS offers the opportunity to analyse enormous and multiple samples simultaneously. In line with this we would like to propose that the above line reads “high throughput sequencing is facilitating ecological and molecular studies by enabling the rapid identification of multiple taxa”.

Reviewer’s comment: 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 one thing in common – they roughly (!) prefer the same kind of food.

Response: Interesting remarks. We will add a sentence or two along the lines of the validity of trophic group classification.

Reviewer’s comment: “for species level identification is vital to accurate and precise computation of nematode indices as determiners of sediment quality” – At species

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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'.

Response: The reviewer is correct. Perhaps a more appropriate way to put this is "for identification to at least the genus level is important for more accurate and precise computation of nematode indices".

Reviewer's comment: "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".

Response: Correction-"species" By sediment, we meant any substrate inhabited by nematodes. Sediment is not the right terminology, since this only refers to aquatic habitats. Correction- "soil"

Reviewer's comment: "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.

Response: We will note the above in our modified manuscript

Reviewer's comments: "... 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.

Response: This will be corrected. We propose removing 'recently'.

Reviewer's comments: "These alternative approaches will undoubtedly rely" – why the two most important ones, crop rotation and host plant resistances, are not mentioned?

Response: We agree that they have to be mentioned since they are among the alternatives we were referring to here. They (both crop rotation and plant resistances) can

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only be effectively implemented if we have knowledge of the plant parasitic nematode (PPN) present in the field. In line with reviewer's comment, we propose the text in the manuscript be replaced with: "For example, such alternative non-chemical approaches as crop rotation and host plant resistance will undoubtedly rely on our knowledge of the taxonomy and biology of plant parasitic nematodes in order to devise efficient and taxa-specific control strategies"

Reviewer's comments: "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).

Response: It is true that these techniques have quite distinct applications. However, all three serve the same general purpose, which is to identify relevant differences between types/species and complement light microscopy. We will remove the phrase "above-mentioned alternatives", since it implies that these approaches can substitute morphology-based identification, which some cannot. It will therefore read, "Each of the above mentioned approaches"

Reviewer's comments: 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)". (. . . I am afraid with quite some overlap with the current manuscript.

Response: The intention was to make mention of some of the identification techniques used in the recent past and these were of course covered in more details in the cited papers above. With your suggested modifications, the final manuscript will have little overlap with the publications we cited above.

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Reviewer's comment: 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.

Response: It is true that there is no reason to try covering the above-mentioned phyla. However, we only mentioned them only for the sake of comparison. The idea was to state how diverse they are compared to other close relatives. We agree that to most specialists, this may seem like an old story. However, SOIL journal is a platform for a range of different readers and therefore the information is of interest.

Reviewer's comment: "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.

Response: We will skip this as suggested by Reviewer.

Reviewer's comment: "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.

Response: We will skip this part.

Reviewer's comment: "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

Response: We will take this part out.

Reviewer's comment: 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

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this.

Response: We will leave this part out.

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).

Response: We will amend this section by just highlighting those parts covered in detail by the above mentioned paper.

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 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.

Response On the section of protein-based methods and ITS, we will shorten the application aspects significantly and only write on some few of their limitations.

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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

Response This is well noted and will be removed from the manuscript.

Reviewer’s comment: 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

Response: Yes, we agree there is currently no “one-for-all” marker. We will include that in this part of the discussion.

Reviewer’s comment: 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 (!).

Response: Yes, we agree it will be worth mentioning some of the factors that can lead to such artefacts forming such as incorrect annealing temperature and cycle number.

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

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

Response: Will consider skipping the Sanger sequencing. We will still review the 454 only by its applications. This is because most application of next generation sequencing

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in nematology to date have been undertaken using this platform.

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