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Can Metabarcoding Override Morphological Approach in Context to Nematode Taxonomy?

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ABSTRACT

Nematodes are one of the diverse groups on earth and act as important biological indicator to monitor soil or marine biodiversity. The common method used to characterize soil or marine nematode community is based on microscopic observations of nematode morphology. Such an approach is time-consuming and requires taxonomic experts. So now a days scientists have started using metabarcoding for rapid identification of nematode community. But there are some disadvantages in molecular analysis for species identification also which mainly lie in the incompleteness of online databases used to assign the taxonomical label to a specific DNA sequence and the need to find the right marker for a specific community. After reviewing different works on study of nematode community structure by morphological approach and metabarcoding it can be concluded that no single method is actually suitable for nematode community analysis, rather an integrated approach to species identification based on morphological and molecular analyses will yield a dataset with even greater reliability than based on only one method.

Keywords: Nematodes, community structure, identification, metabarcoding, morpho-taxonomy

INTRODUCTION

Nematodes are one of the diverse groups on earth and act as important biological indicator to monitor soil or marine biodiversity. Identification of nematodes on basis of morphology has been widely used to assess their diversity but now a days metabarcoding of DNA for bulk samples is increasingly being implemented in ecosystem assessments and it is found to be more cost-efficient and less time-consuming than monitoring based on morphology. With the decline in taxonomic expertise, newly developed molecular methods are being increasingly used in species identification, especially of organisms at the microscopic and microbial scales.[1] Other applications of molecular methods include whole-genome analyses, the determination of evolutionary patterns in phylogeographic and phylogenetic studies and initiatives aimed at collecting the planet's biodiversity in molecular databases.[2-5] Metabarcoding has facilitated studies of small multicellular organisms, either whole communities or specific groups, with marine eukaryotes being a frequent focus.[6-8]

What is Metabarcoding?

Metabarcoding is the large-scale taxonomic identification of complex environmental samples via analysis of DNA sequences for short regions of one or a few genes (called DNA barcodes). It relies on high-throughput DNA sequencing (HTS) technologies, which yield millions of DNA sequences in

parallel and allow large-scale analysis of environmental samples.

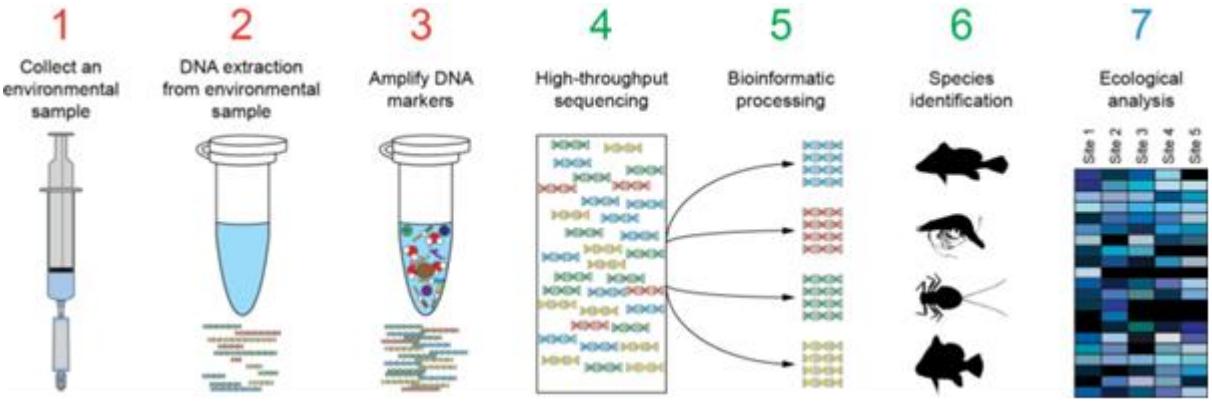

Fig 1. Work flow of metabarcoding
(Source: http://www.naturemetrics.co.uk)

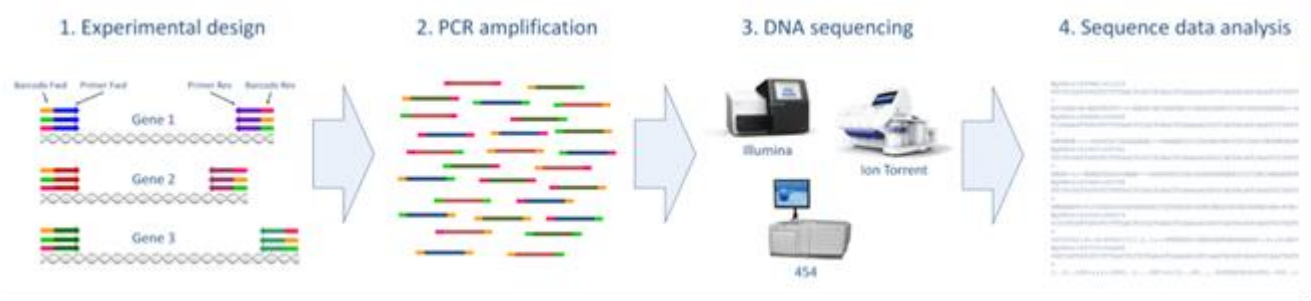

Fig 2. Workflow of high-throughput amplicon sequencing
(Source: http://www.sixthresearcher.com)

With nematodes, it has been found that the COI region designated for most animals lacks the taxonomic coverage (ability to amplify a diverse group of taxa) required of a metabarcoding marker. For that reason, studies on metabarcoding of nematodes thus far have utilized primarily regions within the highly conserved 18S ribosomal DNA.[9] The popular marker within this region is the ones flanked by the primer pair NF1-18Sr2b which is found to be the most suitable both in terms of coverage and ease of access to reference sequences for nematodes.[10]

Metabarcoding has a high resolution for problematic taxonomic groups, as well as specimens at immature stages or specimens that are physically damaged [11-13] and often has a higher specificity to detect nonindigenous species.[14] This shows that metabarcoding allows for inclusion of taxonomic groups in biodiversity analyses that were formerly excluded when using classic monitoring methods.

Metabarcoding in the majority of cases out performed morpho-taxonomy concerning the number of taxa retrieved. [15,13,16] Whilst often finding more taxa, metabarcoding does not necessarily cover the full taxonomic composition found with traditional morphology.

The present study was done to find out whether the newly emerged technique of metabarcoding is fully successful in identifying all the nematodes and can replace the need of classical taxonomy.

MATERIALS & METHODS

A systematic survey of literature was conducted to find out the potentials of metabarcoding in future study of nematode taxonomy. For this, different published articles were searched in google scholar using the key words “DNA metabarcoding of nematodes” and the studies were analyzed.

RESULT

Literature survey shows despite the fact that the use of DNA metabarcoding has been clearly demonstrated in terms of costs and timewise efficiency,[17] its implementation is limited in current monitoring programs due to following clauses as observed by different scientists while their experiment:

Dell'Anno et al. (2015) [18] conducted a study targeting deep-sea nematodes, and found morphological analyses actually resulted in a higher number of nematode species than DNA metabarcoding analysis. It was probably due to an incomplete or inaccurate reference sequence database, which is especially problematic for inconspicuous taxa our relatively understudied species, such as deep-sea nematodes. [18,19] Morphology-based methods may currently still yield better results for the traditionally monitored taxonomic groups.

Panto et al. (2021) [20] suggested HTS offers a faster, more sensitive, less laborious option: however, there is a necessity to improve the coverage of reference sequence databases and the potential limitation concerning thicker nematode's cuticles must be taken into consideration as they potentially lead to underestimations of biodiversity and functional traits. According to these scientists it is premature to completely abandon the traditional taxonomic identification of meiofauna organisms.[20]

To curate global biodiversity in public databases for molecular purposes several initiatives have been attempted but these efforts are far from complete. [21,22] As current databases lack many nematode sequences and a large number of OTUs are of low taxonomic resolution, a taxonomy-free approach might be preferable until alternative measures become available.[23]

Some studies found a good correlation between the proportion of reads and species abundance,[17] sometimes after transformation of the data,[24] majority of studies did not [25,26]and there is still little consensus. So, the main bottleneck of metabarcoding is the lack of correlation between the proportion of reads and the original proportions of species in the sample.

Metabarcoding revealed within family differences in nematode diversity. Metabarcoding over- or underestimated the prevalence of several nematode families compared to morphological analysis, and detected some families that were not observed based on morphology. These differences between the techniques require further investigation to establish the accuracy of metabarcoding for characterization of soil nematode communities.[27]

Ahmed et al. (2019) [10] pointed out that the success of metabarcoding is on the availability of a good reference sequence collection for the marker of choice as well as its taxonomic coverage. The results of his study have shown that despite recommendations to adopt COI-based markers, [28,29] there is still a significant amount of effort needed to make this region a suitable barcode marker for nematodes. This is because the COI region has poor coverage of species and lacks a complete reference database, which make it hard to use for nematode studies, similar to how it's been for other animal groups like birds, fish, and insects.

According to Waeyenberge et al. 2019[30] if the presence of a certain genus or species in monitoring nematode communities is important, one should make sure to optimize the DNA lysis/extraction/purification method to prove that the nematode of interest can be detected. Therefore, in their opinion the metabarcoding is only suitable for monitoring and the results should be treated with caution. His results provided new insights such as the drastic effect of different DNA-extraction methods on nematode species richness due to variation in lysis efficacy.[30]

Macheriotou et al. (2019) [31] curated 18s and CO1 reference sequence databases for species level taxonomic assignments of free-living marine nematodes and pointed

out the scant availability of nematode reference sequence from diverse habitats that hinders a comprehensive characterization of novel ecosystems.

Molecular methods are highly sensitive and may eventually complement traditional taxonomy approaches, but study conducted by Schenk et al. (2020) [32] demonstrate the obvious potential of molecular-based methods to miss species. Their study highlights the need for fundamental work in species identification and the single barcoding of organisms in order to extend and improve current databases. Metabarcoding studies will profit enormously from these efforts, by allowing accurate species- or genus-level identifications. They suggested that an integrated approach to species identification based on morphological and molecular analyses will yield a dataset with even greater reliability than based on only one method. Additionally, future methods, such as whole-genome sequencing and other PCR-free approaches, [5, 33] will eliminate or at least minimize many of the current drawbacks of current molecular approaches, by obviating the need for primers. Through his study Schenk et al. (2020) [32] highlighted the need to quickly expand molecular databases in order to allow the full use of molecular methods in accurate species assignments.

Hayden et al. (2025) [34] discussed the promising potential of molecular based NBIs (Nematode-based indices) as bioindicators of soil health in agricultural and natural ecosystems. But he also pointed out that it is insufficient to assume that metabarcoding community analyses and molecular-based NBIs alone are currently reliable and accurate enough for uses as a bioindicator and discrepancies between nematode community assessment based on morphology and metabarcoding shall always exist.

CONCLUSION

There are several challenges when using DNA metabarcoding to study environmental DNA. The most important of these is the identification of a suitable marker to provide the required taxonomic coverage and species resolution. Another big problem is that DNA metabarcoding relies on PCR, and errors often happen during the amplification process.[35] The way DNA is extracted can also greatly influence how well nematode species are detected, as some methods are more effective at releasing DNA than others. Another difficulty is the need for a large number of DNA sequences from known species. This data collection step is probably one of the most important part, because the accuracy of future identifications depends on how reliable the sequence information in the database is. Without any sequence from described taxa to match the obtained sequences with, they may convey limited biological or taxonomic meaning to the investigator.

The first studies on the prospective use of DNA-metabarcoding to characterize nematode communities showed some shortcomings which include failure to detect a number of nematode species leading to underestimation of species richness. In some other cases it picked up variations within the same species increasing the number of OTUs (operational taxonomic units) or ‘molecular’ species, and cause flaws in quantification. So the results should be treated with caution and the technique is only suitable for monitoring. Nematode quantification using microscopy is assumed to be more reliable than for molecular analyses. Whilst often finding more taxa, metabarcoding does not necessarily cover the full taxonomic composition found with traditional morphology.

Now a days DNA metabarcoding of bulk samples is becoming more common to assess ecosystem as it is less time-consuming and more cost-efficient than monitoring based on morphology. DNA metabarcoding still has some real challenges. Some of these are inherent constraints of the techniques used and are unlikely to completely disappear. Classical morpho-taxonomy is the most authentic

method till date to identify nematode species. So morpho taxonomy should not be abandoned. Instead, integrated approach of metabarcoding and morpho taxonomy can provides accurate data about nematode community structure. So, both the approaches should be adopted together for study of nematode community analysis. Crisis of taxonomists is of great concern nowadays so young researchers should be encouraged to learn morpho taxonomy.

Declaration by Authors

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