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Exploring the use of metabarcoding to reveal eukaryotic associations with mononchids nematodes

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Abstract

Nematodes play a vital ecological role in soil and marine ecosystems, but there is limited information about their dietary diversity and feeding habits. Due to methodological challenges, the available information is based on inference rather than confirmed observations. The lack of correct dietary requirements also hampers rearing experiments. To achieve insight into the prey of mononchid nematodes, this study employed high-throughput Illumina paired-end sequencing using universal eukaryotic species 18S primers on 10 pooled mononchid nematode species, namely *Mylonchulus brachyuris, M. brevicaudatus, Mylonchulus* sp., *Clarkus parvus, Prionchulus* sp. *M. hawaiiensis, M. sigmaturellus, M. vulvapapillatus, Anatonchus* sp. and *Miconchus* sp. The results indicate that mononchids are associated with a remarkable diversity of eukaryotes, including fungi, algae, and protists. While the metabarcoding approach, first introduced here for mononchids, proved to be a simple and rapid method, it has several limitations and crucial methodological challenges that should be addressed in future studies. Ultimately, such methods should be able to evaluate the dietary complexity of nematodes and provide a valuable avenue for unraveling the dietary requirements of previously unculturable nematodes. This can contribute to the methodology of understanding their feeding habits and contributions to ecosystem dynamics.

Keywords

18S, dietary, ecological, metabarcoding, method, sequencing, universal

Mononchid nematodes (Mononchida Jairajpuri, 1969) are a common order of predatory animals in soil. They have enveloping and swallowing feeding organs that allow them to feed on invertebrates such as rotifers, enchytraeids, protozoa, and other nematodes (Cobb, 1917). Most studies on mononchids mainly focus on species identification (Tahseen et al., 2013), occurrence in ecological studies (Yeates et al., 1993), and their role as biocontrol organisms to plant-parasitic nematodes (Bilgrami, 2008). However, despite their importance, there are very few studies on the feeding habits of mononchids. Understanding their feeding habits not only aids comprehension of their position and role in the soil ecological environment (Ferris et al., 2001) but also helps in improving artificial rearing for biological control research (Salinas & Kotcon, 2005).

Early studies on mononchid feeding behavior and their food intake relied on 1) observing gut contents (Bilgrami et al., 1986); 2) observations from in vitro experiments (Yeates, 1969; Small & Grootaert, 1983); and 3) observations from pot experiments using defined specimens of preys and predators (Small, 1979; Khan & Kim, 2005). However, these traditional short-term experimental observations had limitations in accurately determining the composition of ingested food due to the long life-cycle of mononchids, which led to prey degradation. Similarly, use of species-

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specific primers to target DNA of plant-parasitic nematodes in the gut of excised predatory nematodes (Cabos et al., 2013) was also suboptimal as only the presence or absence of the plant-parasitic nematode prey DNA could be reported.

However, with advances in sequencing techniques, novel metabarcoding tools have become valuable for studying the food spectrum of various organisms. Metabarcoding tools can identify highly degraded food fragments, thereby revealing a more comprehensive food diversity in organisms (Pompanon et al., 2012). Currently, this technique has been applied to various diet assessments, e.g., in insects (Pons, 2006), herbivorous birds (Valentini et al., 2009), and mammals (Soininen et al., 2013; Deagle et al., 2009).

While metabarcoding has been used extensively in other biological groups, its application in terrestrial nematodes has been relatively limited and mainly used for nematode community profiling with a focus on primer efficiency comparison, species identification, and community characteristic analysis (Porazinska et al., 2009; Waeyenberge et al., 2019). However, some recent studies have examined nematode-associated organisms using metabarcoding. For instance, Schuelke et al. (2018) analyzed the microbial communities associated with marine nematodes and their relationship to host phylogeny, geographic region, or feeding morphology. Additionally, McQueen et al. (2022, 2023) compared the internal and external microbiomes of omnivorous *Eudorylaimus antarcticus* and bacterivorous *Plectus murrayi* using 16S and 18S rRNA. They noted that the gut microbiomes exhibited lower diversity and were compositionally different from the microbial community of their habitat. However, since the known microbiomes of nematodes are presently restricted in number, it is imperative to investigate additional nematode species and analyze their gut composition. This information not only aids in enhancing our comprehension of nematode roles in ecosystem processes but also proves highly valuable in rearing studies, particularly when attempting to culture nematodes that have not been previously cultured, such as many enoplids and dorylaims. Fortunately, metabarcoding tools provide a way to obtain such information. In the present study, we use metabarcoding-generated amplicon sequences of the 18S rRNA gene to evaluate their effectiveness in discerning the composition of eukaryotic communities across ten mononchid nematode species representatives. We hypothesize that the generated ASVs will provide a sufficient community profile of gut-associated species within the studied mononchid species.

Materials and Methods

Sampling and nematode extraction

Mononchid populations (juvenile stages) were obtained from soil samples collected at six geographical locations (Table 1) in China. Nematodes were extracted by placing 200g of soil on a modified Baermann tray for 24 h at 25°C and recovered using a 50 µm sieve (Whitehead & Hemming, 1965). Thereafter, nematodes were individually picked under a dissecting microscope (Motic SMZ160) and underwent three sequential washes with sterile water to eliminate cuticular organisms, as outlined and validated in Derycke et al. (2016).

Table 1: Sampling information of Mononchid populations used in this study.

Species	Habitat	Sampling locality
M. brachyuris	Grass land soil	Linzhi, Tibetan
C. parvus	Moss soil	Zhouzhi, Shaanxi
Miconchus sp.	Moss soil	Nanjing, Jiangsu
Prionchulus sp.	*Populus* sp. rhizosphere	Qiqihar, Heilongjiang
M. brevicaudatus	Moss soil	Zhouzhi, Shaanxi
M. hawaiiensis	Moss soil	Nanjing, Jiangsu
M. sigmaturellus	*Digitaria* sp. rhizosphere	Nanyang, Henan
M. vulvapapillatus	Moss soil	Hulun Buir, Inner Mongolia
Mylonchulus sp.	*Populus* sp. rhizosphere	Qiqihar, Heilongjiang
Anatonchus sp.	Moss soil	Zhouzhi, Shaanxi

DNA extraction for associated eukaryotic species

First, nematode identification was done morphologically, whereby 1-2 of the washed nematodes were mounted on temporary slides and examined microscopically using the Olympus BX optical microscope. Thereafter, DNA was extracted using freeze-thaw extraction method (Mikaeili et al., 2013) from the 10 mononchid species. Briefly, 8 µL of distilled water with individual nematodes in a 0.2 mL PCR tube was placed in liquid nitrogen and frozen for 2 min, quickly taken out, and placed in a 65°C water bath to melt for 1 min. These steps were alternately performed three times to make sure that the nematodes were fully broken. 50 µl 10× PCR buffer and 1 µL of 100 mg/µL proteinase K solution were added to the PCR tube, shook, mixed well, and subsequently incubated in a thermal cycler for 90 min at 65°C and 10 min at 95°C to obtain a crude DNA extract.

Amplification of 18S fragment of rRNA

Three independent PCRs were conducted, and the resulting products from all three were combined together as one sample for the subsequent sequencing step. We used two sets of PCR primers that specifically target the eukaryote V4 and V1-3 regions of the 18s rRNA. For the V4 region, the primers were TAReuk454FWD1 (5´-CCA GCA SCY GCG GTA ATT CC-3´) and TAReukREV3 (5´-ACT TTC GTT CTT GAT YRA-3´) (Stoeck et al., 2010). For the V1-3 region, the primers were EUK20f (5´-TGC CAG TAG TCA TAT GCT TGT-3´) and EUK302r+3 (5´-ACC AGA CTT GYC CTC CAA T-3´) (Euringer & Lueders, 2008). We used a two-step PCR method to add sample-specific indices. The reaction mixture of the first step PCR consisted of 1 µL of nematode DNA template, 2 µL of forward and reverse primers with a concentration of 10 µM, 2 µL of 100 µM dNTPs, 2.4 µL of 10×ExTaq PCR buffer, 2 µL of 25 mM $MgCl_2$, 0.1 µL of 5 U/µL ExTaq enzyme, and ddH_2O added to make up to 25 µL. The reaction conditions were 95°C for 4 min; 30 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min; and total extension at 72°C for 10 min. In the second step of PCR, the first step PCR amplification product was diluted 10 times in sterile water and used as the template, and distinct samples were amplified using primers tagged with different barcodes, permitting sample discrimination after MiSeq sequencing. The PCR mixture was the same as above, and the reaction conditions were 95°C for 4 min; 95°C for 30 s, 54°C for 30 s, 72°C for 1 min, cycle 25 times; and 10 min extension at 72°C.

Library preparation and high-throughput sequencing

The Cycle-Pure Kit purified the ten 18S fragment products, and DNA was quantified using the Qubit® 1× dsDNA HS Detect Kit (Yeasen Biotech, Shanghai, China) on the Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, USA), then mixed in equal amounts (100 ng per sample) to obtain a mixed pool. The genomic library preparation was performed using the Illumina TruSeq DNA Sample Preparation Kit (Illumina, Inc, USA). The paired-end sequencing was performed on Illumina MiSeq 2 × 300-bp platform, and sequencing work was completed by Shanghai Biozeron Co., Ltd.

Bioinformatics analysis

Quality control was performed on the obtained raw data using fastp (Chen et al., 2018), and the reads with Q<20 were removed. The adapters were identified and removed using cutadapt (Martin, 2011). The processed sequence data were imported into the QIIME2 software (Hall & Beiko, 2018). The DADA2 (Divisive Amplicon Denoising Algorithm) (Callahan et al., 2016) algorithm was applied with default parameters to conduct quality control, noise reduction, and chimera removal on the sequences, to obtain amplicon sequence variants (ASVs) and optimized sequences. The obtained ASVs were assigned taxonomy using the Bayesian algorithm of the RDP classifier (http://rdp.cme.msu.edu/) and searched against SILVA (https://www.arb-silva.de/) database version 138, using a confidence threshold of 0.7.

Results

Amplification, high-throughput sequencing, and generation of ASVs

Fragment sizes of about 450 and 500 bp were obtained after successful amplification when using primer TAReuk454FWD1/TAReukREV3 and EUK20f/EUK302r+3 (Figs. 1A and 1B). A total of 304,751 raw reads were generated from the pooled samples by Illumina sequencing. After quality control from the cutadapt package, we obtained 184,429 sequences. Following quality control, denoising, and chimera removal, 157,297 reads remained, which were then demultiplexed into 10 samples using DADA2 (Table 2). The average amplification efficiency of EUK20f/EUK302r+3 primer was much higher than that of the TAReuk454FWD1/TAReukREV3 primer.

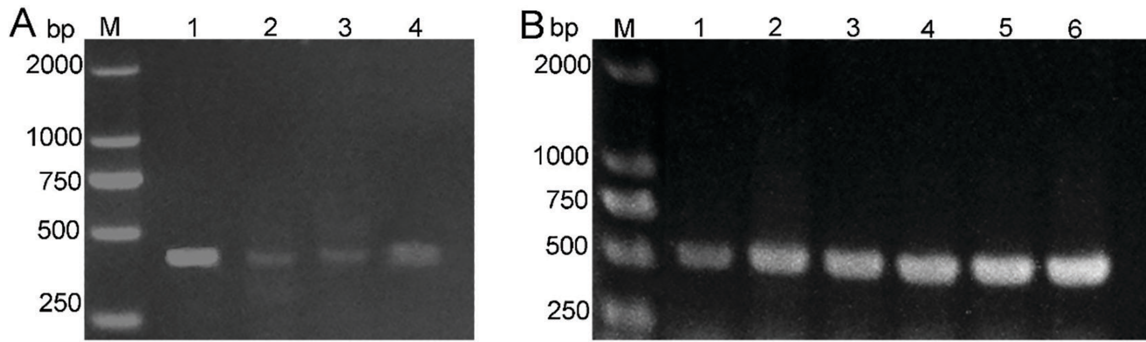

Figure 1: Detection of amplification fragments of rDNA 18S from 10 Mononchid species. A: Amplification with primer pair TAReuk454FWD1/TAReukREV3, lines 1-4 are *M. brachyuris, C. parvus, Miconchus* sp., and *Prionchulus* sp. respectively; B: Amplification with primer pair EUK20f/EUK302r+3, lines 1-6 are *M. brevicaudatus, M. hawaiiensis, M. sigmaturellus, M. vulvapapillatus*, *Mylonchulus* sp. and *Anatonchus* sp. respectively. M: DNA marker DL2000.

Table 2: Primer pair used, generated reads, and ASVs per mononchid sample.

	Reads				
Species	**Kingdom**	**Phylum**	**Order**	**ASVs**	**Primer used**
M. brachyuris	43	43	43	2	TAR
C. parvus	42	42	36	3	TAR
Miconchus sp.	2317	2315	2315	6	TAR
Prionchulus sp.	214	214	211	3	TAR
M. brevicaudatus	5983	5983	3618	7	EUK
M. hawaiiensis	43008	42672	40617	34	EUK
M. sigmaturellus	31737	30422	28781	89	EUK
M. vulvapapillatus	3944	3944	3929	13	EUK
Mylonchulus sp.	34869	34863	34864	15	EUK
Anatonchus sp.	35140	35140	34959	30	EUK

ASVs (Amplicon sequence variants). TAR (TAReuk454FWD1/TAReukREV3). EUK (EUK20f/EUK302r+3).

Taxonomy assignment

To identify the eukaryotic species associated with the 10 mononchid species, a total of 202 ASVs were annotated using the SILVA taxonomic framework (Yilmaz et al., 2014). Taxonomy assignments were made at three levels, including kingdom, phylum, and order. At the kingdom level, a total of 157,297 reads were assigned to eight groups. The most dominant kingdoms were Animalia, mostly because of targeting the mononchid host, Fungi, and Plantae. At the phylum level, a total of 155,638 reads were assigned to 15 phyla (Fig. 2), with Nematoda being the most dominant group in all samples (86-100%). At the order level, a total of 149,373 reads were assigned to 18 groups (Fig. 3). As expected, the most dominant group in all samples was mononchid reads from the host. Remarkably, there was a consistent absence of DNA from non-mononchid nematodes across all samples (Figs. 2,3). Several species were assigned a remarkably low number of taxa at each of the three hierarchical levels: kingdom, phyla, or order. These species include *Mylonchulus brachyuris, Mylonchulus brevicaudatus, Mylonchulus* sp., *Clarkus parvus,* and *Prionchulus* sp. (Figs. 2,3). In these species, mononchid DNA (Nematoda; 86-100%) was dominant (Figs. 2,3); followed by protists (Cyrtophorida, Ciliophora; 14.2%); and yeasts (Moniliales, Ascomycota; 6.6%), which were only present in *C. parvus* and *M. brevicaudatus,* respectively (Fig. 3). Stramenopile algae (Ochrophyta) were found to be a relatively important component

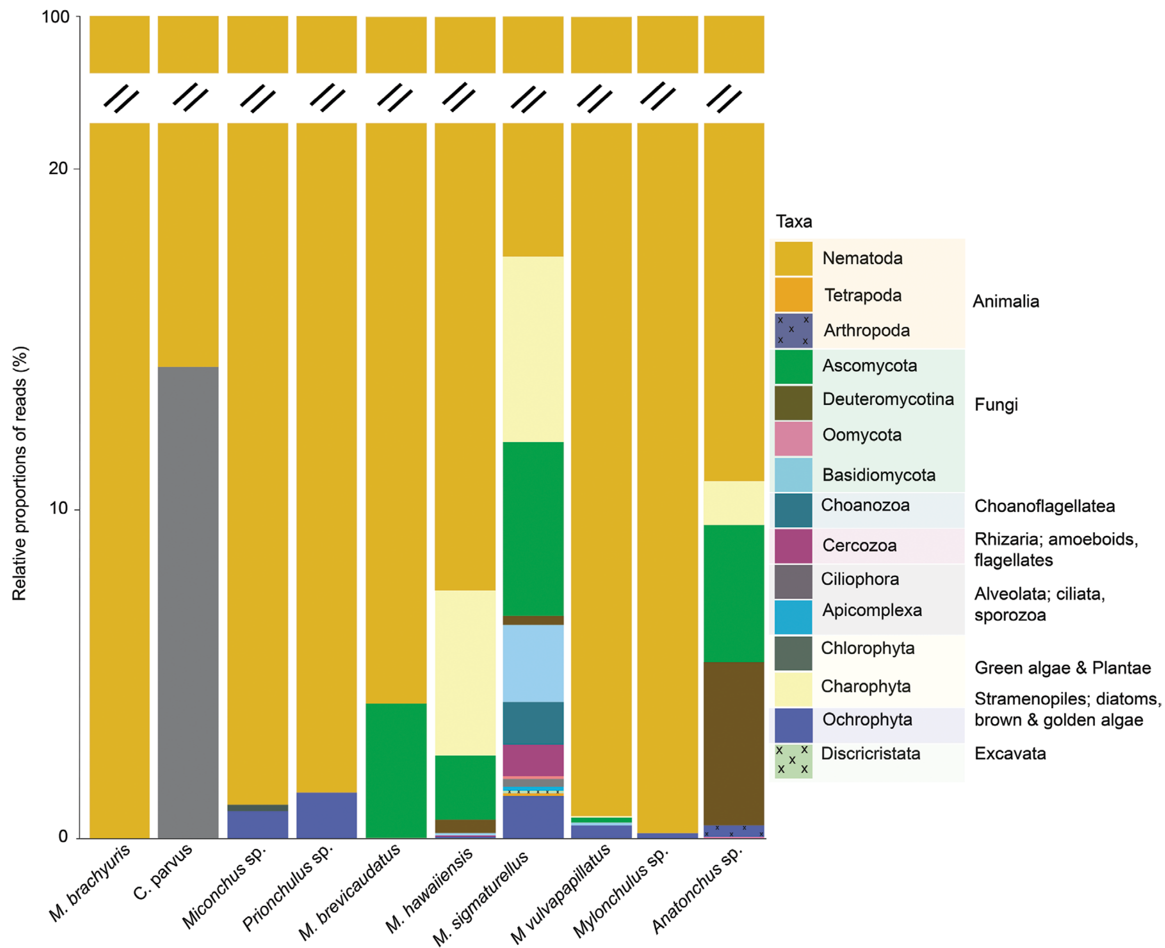

Figure 2: Diversity of eukaryotic organisms presented in 10 mononchid species at phylum level. The classification system is based on the eukaryote classification system of Adl et al., (2012).

within three of the mononchid taxa studied, albeit in low proportions (0.15-1.4%) (Fig. 2). These algae were predominantly represented by golden algae (Ochromonadales and Chromulinales; Fig. 3).

The five remaining mononchid species (*M. hawaiiensis, M. sigmaturellus, M. vulvapapillatus, Anatonchus* sp., and *Miconchus* sp.) were associated with a broader range of eukaryotic groups. However, even in these species, mononchid DNA (Nematoda; 87-99%) was dominant, while the other non-mononchid taxa had a relatively low proportion (1-13%) (Figs. 2,3). The most dominant non-mononchid taxa were fungi, including Deuteromycotina (0.3-5%) and Ascomycota (0.2-5.3%), which were obtained in 3 and 4 of the species, respectively (Fig. 2). Specifically, Ascomycota were mainly represented by Moniliales (1.7-5%), and Deuteromycotina represented by Malasseziales (0.7-0.8%), both present in two of the mononchid taxa (Fig. 3). The other commonly observed taxa included green and stramenopile algae (Charophyta: 0.05-5.7% and Ochrophyta: 0.01-0.8%), both obtained in 4 of the 5 mononchid specimens (Fig. 2). Stramenopile algae were mainly presented by golden algae (Ochromonadales: 0.1-0.4% and Chromulinales: 0.8%) (Fig. 3).

In addition, ciliates (Ciliophora: 0.2-14%) were also a relatively important part of the associated taxa, albeit obtained in only two of the 10 mononchid taxa studied (Fig.2). Other taxa associated with the mononchid nematodes included Rosales (Plantae; 1.7%) (Fig. 3) and Tetrapoda (Animalia; 0.06%) (Fig. 2), found only in *M. hawaiiensis* and *M. sigmaturellus*, respectively. Finally, Solanales and Asparagales (Plantae; 0.01-4.7% and 0.1-4.2%, respectively) were found in 3 and 2 of the total 10 mononchid taxa studied (Fig. 3). Relative proportions of the taxa found, after excluding all nematode reads, are visualized in a supplementary figure (Figs. S1).

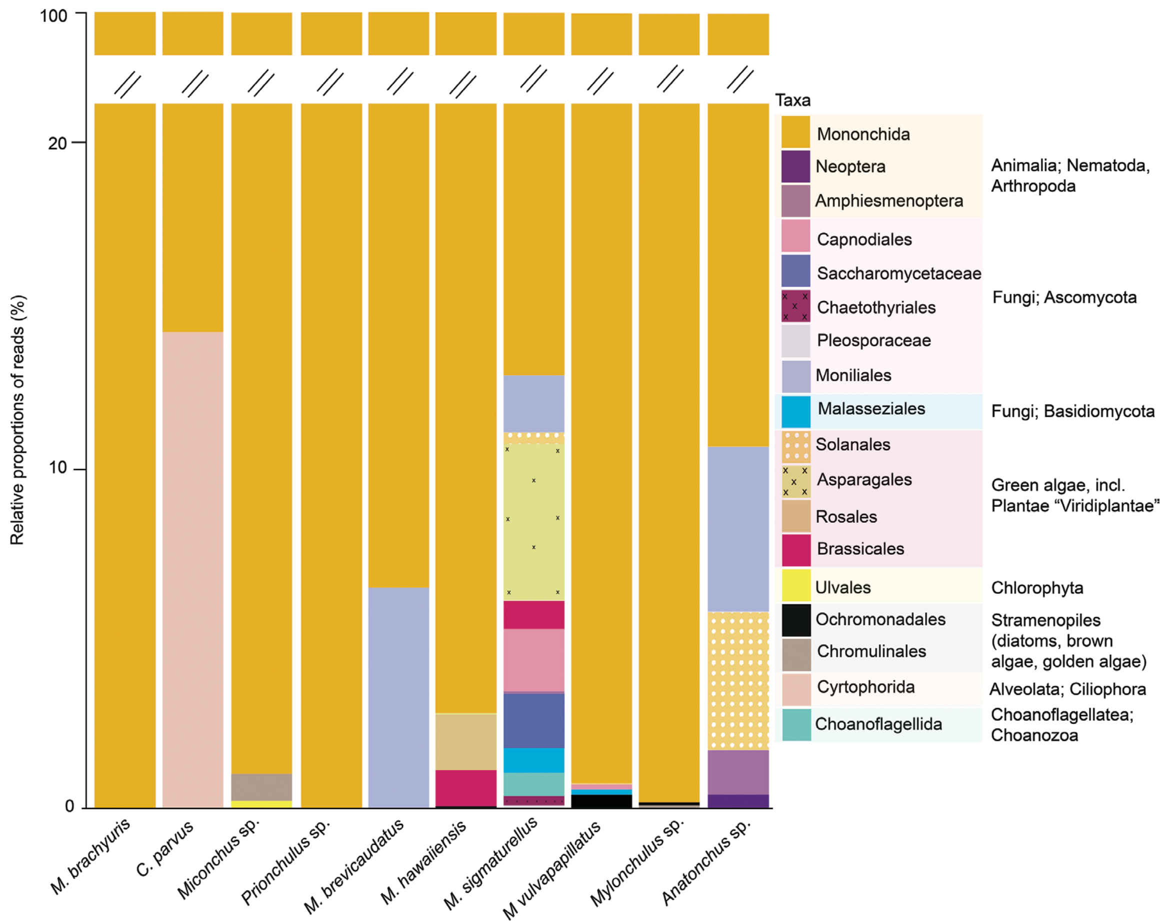

Figure 3: Diversity of eukaryotic organisms presented in 10 mononchid species at order level. The classification system is based on the eukaryote classification system of Adl et al., (2012).

Discussion

To date, mononchids have been classified as carnivorous based on their buccal morphology (Cobb, 1917; Yeates et al., 1993). However, it has been well established that they also utilize other food sources (Yeates, 1987; Salinas & Kotcon, 2005). In fact, the determination of nematode feeding habits has mostly been "inferred rather than confirmed" through long-term observation under specific biological conditions (Yeates et al., 1993). Observation of bacteria in the intestines of *Clarkus papillatus* and *Prionchulus muscorum* (Arpin & Kilbertus, 1981) indicated that certain bacteria present in their intestines could serve as potential food sources for these mononchids. Salinas & Kotcon (2005) suggested that juveniles of *C. papillatus* survive on bacteria and dead or wounded prey that adults of their species previously attacked. Furthermore, *M. propapillatus* has been observed to thrive on large quantities of bacteria (Yeates, 1987). These findings highlight the arbitrary nature of traditional nematode feeding group classification and suggest a more flexible approach when considering certain ecological factors.

Previous studies have established that subjecting nematodes to a series of washing steps reduces organisms that might adhere to the cuticle (Berg et al., 2016; Derycke et al., 2016; Schuelke et al., 2018), and therefore, we speculate that the recovered eukaryotes are "gut-associated". These "gut-associated" eukaryotes may have been acquired in one of the following ways: (1) directly derived from the organisms ingested by the mononchid nematodes, (2) indirectly derived from the food sources of the ingested organisms, or (3) indirectly obtained from water and soil containing plant DNA that was passively ingested. Although the limited resolution of taxonomic categories was attained, we obtained a significant overlap in associated taxa across the different mononchid species at the order level, which represents our lowest achievable

taxonomic resolution. Consequently, we posit that it is plausible to assume that the observed taxa might reasonably reflect the actual dietary items rather than just mere contaminants. For example, Fungi, particularly Ascomycota, were found to be associated with four mononchid nematode species, and Deuteromycotina and Basidiomycotina were also observed in most mononchid taxa. However, more precise data on fungi as a food source for nematodes are predominantly limited to fungal-feeding nematodes. For example, Ruess et al. (2002) demonstrated that *Aphelenchoides* sp. contained several fatty acids known from the various types of ascomycetes, basidiomycetes, and mitosporic fungi used in the rearing studies. Moreover, rRNA metabarcoding analysis of the gut microbiome of both the omnivorous *Eudorylaimus antarcticus* and bacterivorous *Plectus murrayi* revealed the presence of ascomycetes such as *Tetracladium furcatum* and basidiomycetes (McQueen et al., 2022). Thus, it is likely that the fungal taxa identified in our study are either directly ingested by the mononchid nematodes, contributing to their diet, or indirectly derived from the prey organisms' food sources, considering the predatory behavior of mononchids. Similarly, green algae (Charophytes) and golden-brown algae (Ochrophytes) were found to be associated with most mononchid nematode species, which aligns with limited evidence of terrestrial nematodes consuming algae, albeit only in specific groups. For example, *Aporcelaimus* sp. and *Aporcelaimellus* sp. have been observed feeding on specific green algal taxa, such as *Haematocococcus*, *Microcoleus*, and *Chlorella* sp. (Wood, 1973). Similarly, Schuelke et al. (2018) reported the presence of Charophyta, Chlorophyta, and Ochrophyta, among others, as part of the microbiome of certain marine nematode species. Hence, some of the identified algal taxa likely constitute dietary items for mononchids.

However, other DNA sequences obtained, such as those from flowering plants (Rosales), could not be readily explained and may have been indirectly obtained from food sources of the ingested organisms (2) or water and soil containing plant DNA that was passively ingested (3). It is unlikely that mononchids would consume prey without simultaneously ingesting plant material, as evidenced by the green pigmentation observed in the intestines of many mononchids and dorylaims (Cobb, 1917), suggesting a possible plant or algal origin. Similarly, the presence of tetrapod (limbed vertebrates) DNA in *M. sigmaturellus* is probably due to contamination from environmental or human sources. While the sources of contamination remain speculative, we suggest that future studies incorporate environmental samples into their analyses. This approach will facilitate comparisons, and discrepancies can be confidently validated.

Surprisingly, we failed to recover nematode species other than mononchids. This is consistent with our observation that no body remains of nematodes were found in our analyzed mononchid nematodes. However, this contradicts previous records that suggest mononchids prey on a wide variety of nematodes including *Pratylenchus, Hoplolaimus* and *Tylenchorhynchus,* as observed from the intestinal contents of certain mononchid nematodes such as *Mononchus aquaticus* (Bilgrami et al., 1986). Several reasons may explain the absence of nematode recovery. (1) The nematode predatory behavior is stage-specific, e.g., only in the adult stage when teeth are well-developed. At the same time, in this study, only juveniles were investigated because adults were rare in the Mononchid populations. (2) The nematode predatory behavior is environment-related. Our tested mononchids were sampled from November to February when soil temperature is generally lower than 10°C, and predatory behavior may be interrupted by the low temperature. (3) Nematode prey can be digested or degraded faster than plant or fungi food sources. Further experiments are needed to examine the intraspecific and temporal variation on gut content, as well as the sensitivity of recovery at different digestion time points. In particular, sequencing individual nematodes instead of pooled samples and sequencing the same nematode species collected from different locations could answer these questions while distinguishing between real prey versus organisms associated only with the predator. It is therefore evident that the technique employed in this study is not conclusively validated. Therefore, we refrain from making definitive statements about the extent to which the observed diversity of organisms accurately represents the actual composition of the diet of the studied mononchid nematodes.

Nevertheless, despite its limitations, the metabarcoding approach utilized in this study represents an initial effort to unravel associated taxa that could serve as food sources for nematodes, followed by employing alternative methods to validate the genuine diversity of the recovered taxa. This is particularly valuable for nematodes that cannot be cultured or are fluid feeders, where direct observation of food intake or analysis of gut content is difficult or even impossible (Small, 1987). The feasibility of detecting and analyzing remnants of fluid-based or partially degraded food materials

in these nematode species could only be achieved through the implementation of such an approach as outlined in this study. Additionally, for nematodes that can potentially be cultured, our understanding of the nutritional needs of these (aquatic) nematodes remains limited (Moens & Vincx, 1998). Feeding experiments based on the revealed diet from the metabarcoding approach, while taking into account the necessary experimental improvements, could provide further insights into this matter.

Therefore, while metabarcoding is certainly a promising method for determining the "gut-associated" taxa of mononchid nematodes, several caveats should be considered. First, the universal eukaryote primers used in this study were designed to target a highly conserved region of the SSU DNA (V4 and V1-3), resulting in a wide range of taxa but with limited resolution. Consequently, the inability to identify taxa beyond the order level posed a challenge that made it impossible to fully answer specific dietary questions. For example, investigations into the possible consumption of other nematode taxa by the mononchids remained unresolved in our study. Thus, although an optimal primer set should reliably amplify DNA from a wide array of ingested food sources and provide an optimal resolution, all barcode primer sets developed so far entail certain trade-offs in addressing this scenario, as exemplified in this study and elsewhere (Pompanon et al., 2012). Another challenge was the primers' inability to distinctly amplify preys' DNA, resulting in a very high proportion of reads (86% to 100%) originating from mononchid nematodes themselves. To address this, future studies should attempt to limit the amplification of predator DNA by incorporating a blocking oligonucleotide in the same PCR (Vestheim & Jarman, 2008; Deagle et al., 2009). Alternatively, species-specific or group-specific primers designed to amplify target prey while discriminating predator's DNA can be used (Vestheim & Jarman, 2008; King et al., 2010). However, the latter approach is problematic because designing non-universal primers while targeting a diverse community can be challenging (Vestheim & Jarman, 2008). Furthermore, it is currently impossible to detect DNA resulting from cannibalism as prey and predator DNA from the same species cannot be distinguished using current techniques (Shehzad et al., 2012).

Secondly, quantifying taxa based on the proportion of obtained sequences remains problematic. Metabarcoding-based approaches only indicate the presence and frequency of target DNA sequences in the sample, which does not correspond to the actual proportional biomass of ingested items (Harper et al., 2005; Casper et al., 2007). Additionally, DNA sequence quality can be affected by biological processes such as digestion while inside the host (Deagle et al., 2009) and PCR bias during amplification (Engelbrektson et al., 2010). Therefore, estimating the extent to which obtained DNA sequences reflect the actual relative food biomass requires feeding nematodes with a mock diet (diet of known proportions), which was beyond the scope of this study.

In summary, the results of the described metabarcoding approach underscore crucial methodological challenges that need to be addressed in future studies. A key concern is the need to differentiate between surface attached microbes that are possibly not effectively removed during the washing step, tissue symbionts, and actual gut contents. A possible solution would be to extract total genomic DNA content from the dissected nematode intestines rather than inferring diversity from entire organisms. Ideally, such an experiment could focus on individual predatory nematodes with observed prey remains in their digestive system. Alternatively, species of mononchs that can be grow on agar plates with different prey species can be used. In this way, the sensitivity and reliability of the metabarcoding approach used for identifying observed prey can be effectively tested. We, therefore, recommend more empirical efforts to improve metabarcoding tools for accessing the food diversity of nematodes before making any definitive assumptions about their dietary composition. Nevertheless, this study introduces for the first time a simple and rapid approach for evaluating the food intake diversity of mononchid nematodes despite several limitations.

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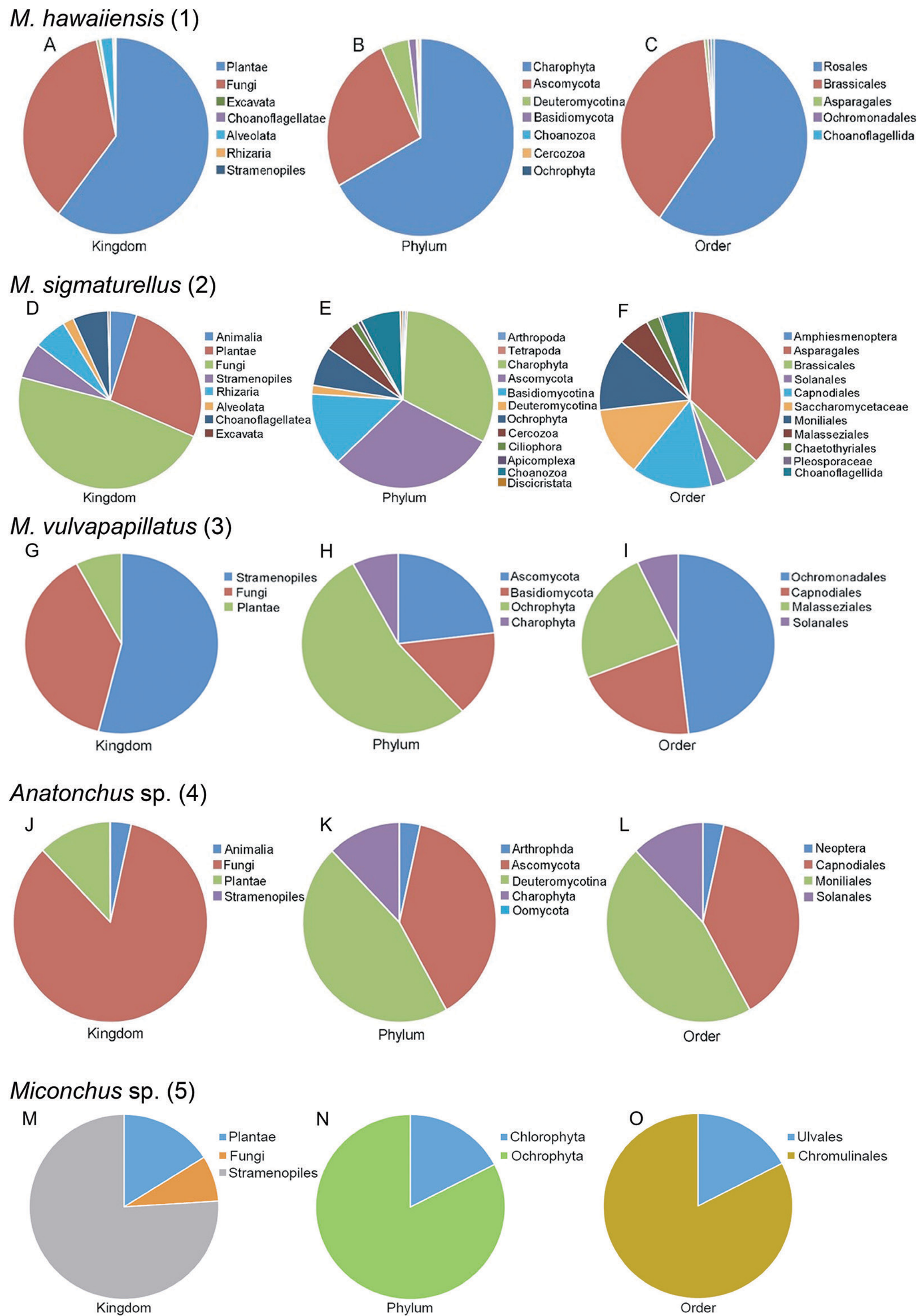

Figure S1: Relative abundance of eukaryotic organisms presented in 10 mononchid species at different taxon levels. The percentages of each of the phyla or orders are calculated over the total number of reads excluding the nematode reads.