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Article

Discovery of Beetles in the Diverse Diets of Water Mites in a Vernal Pond Using Next Generation Sequencing

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Abstract

Vernal ponds are temporary, isolated bodies of water that lack vertebrate predators like fish. Palmer Park in Detroit MI USA, contains an old-growth forest with multiple vernal ponds that are home to numerous invertebrates, including water mites, which are the main focus of this study. These vernal ponds are unique since they are geographically isolated in the middle of an urban landscape. Previous research discovered new species of non-biting midges in Palmer Park vernal ponds, suggesting the potential for the discovery of previously undocumented organisms and relationships in this ecosystem. Here we document the invertebrate species found in the vernal ponds of Palmer Park, both to illustrate their diversity and to determine their cytochrome oxidase I barcodes. The barcodes are used in this study to verify identification and to provide reference sequences for comparison to sequences in the diets of water mites also collected from the ponds. Three taxa of water mites found in Palmer Park Pond A are *Hydryphantes waynensis*, *Parathyas* sp., and *Hydryphantes* sp. (distinct from *H. waynensis* by having a COI barcode 11.6% different from *Hydryphantes* sp.). This paper also uses Next Generation Sequencing (NGS) to analyze the complex diets of the water mites at Palmer Park. The diets consisted of a diversity of species of worms, mosquitoes, non-biting midges, crustaceans, flies, and beetles. Beetles identified as whole organisms in the ponds or from diet-detected bar codes in vernal pond water mites include *Copelatus glyphicus*, *Acilius* sp., and *Hygrotus sayi.* The diets of water mite in these vernal ponds are compared to previous molecular studies in which water mites in a riverine lagoon were identified as opportunistic predators of a diverse invertebrate diet. Beetles have not previously been reported in water mite diets, so this finding represents a new discovery. Diet analysis revealed taxa and novel barcodes not observed through traditional sampling, highlighting the value of water mites for community characterization. These results support the hypothesis that water mites are opportunistic predators, uniquely reports beetles in their diets, and emphasizes their ecological importance and utility in assessing vernal pond biodiversity.

Keywords: beetles; cytochrome oxidase I barcoding; *Hydrachnidia*; *Hydryphantes*; *Parathyas*; predator; prey; vernal pond

1. Introduction

Vernal ponds are temporary bodies of water, not connected to flowing rivers or streams, and are filled annually with rainwater and snow melt [1]. These ephemeral ponds provide an important

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habitat for biodiversity and are beneficial for flood control, filtration, and nutrient cycling [2]. Vernal pond communities lack fish but typically include frogs and salamanders, and numerous invertebrates [3], including water mites, which are the main focus of the present study. Other invertebrates typically found in vernal ponds include caddisflies, fairy shrimp, clam shrimp, tadpole shrimp, damselflies, dragonflies, chironomid larvae, beetle larvae, mosquito larvae, water fleas, copepods, ostracods, and worms [3]. A general challenge for understanding the ecology of ephemeral ponds is determining the predator-prey relationships and foraging strategies among diverse organisms in this stressful varying habitat. Predator and prey may be only intermittently present and must undergo rapid development due to the short duration and periodic, somewhat unpredictable rise, fall, and drying of the aquatic environment.

Here, we specifically focus on the diets of water mites, whose adults typically prey on the types of small aquatic invertebrates such as aquatic insect larvae, ostracods, and water fleas [4,5], that are found in vernal ponds. Water mite diets in this distinctive environment have not been extensively studied. In vernal ponds, they may have unique diets, especially as they lack competition from fish [2], in comparison to the fish-containing lotic river and lentic lake and lagoon environments that are the typical focus of water mite studies. Knowledge of the diets of water mites can provide important information on ecosystem diversity and species relationships. In fact, a study of the diets of water mites in a lagoon environment using molecular methods revealed a generalist diet that not only included the expected chironomid and crustacean DNA markers in water mite guts but also a diversity of oligochaetes, which were not previously known as water mite prey [5]. Indeed, water mite predation seemed to have mined a "hidden biodiversity" of sequences that indicated numerous insects, and, especially, oligochaetes that were present in the environment but not yet molecularly described to species level. We hypothesized that the study of water mites in vernal ponds would similarly reveal previously unknown predator-prey relationships and potentially unsuspected or undescribed inhabitants of the vernal pond environment.

Like other arachnids, water mites prey upon other organisms by piercing their integument with mouthparts, possibly injecting toxins and enzymes into their prey organisms, and sucking liquified partially digested nutrients (including prey DNA) into their gut, where it may be further digested [4,6,7]. Prior to a preliminary laboratory study on DNA recovery from chironomid larvae that had been preyed upon by *Hyrgrobates fluviatilis* water mites [8], most information on water mite prey came from laboratory feeding experiments. These experiments would offer various prey to hungry water mites, and observations were made on what organisms the water mites would settle on, pierce, and ingest. Typical prey items, identified primarily through laboratory feeding experiments and summarized by [4], included insect larvae of chironomids [9] and mosquitoes [10]; ostracods [11–13], and cladocerans [14].

Various field studies support water mite diet observations. Water mite predation caused a 50% decline in over-wintering larvae of a chironomid species in a Dutch lake [9]. Zooplankton decreased in response to an invasion of a shallow tropical lake by *Krendowskia* in Brazil [15]. The richness and abundance of water mites in Croatian reservoirs have strong correlations with a combination of prey items that included chironomids, cladocerans, copepods, and ostracods [16].

Martin et al. (2015) showed that DNA of chironomids of subfamily Tanytarsini ingested by *H. fluviatilis* water mites in the lab can be detected up to 24 hours after ingestion. Molecular methods were used by the Ram laboratory to analyze the gut contents of freshly collected water mites from Blue Heron Lagoon, an arm of the Detroit River on Belle Isle in Detroit, MI USA [5,7,17]. Based on cytochrome oxidase I (COI) molecular barcodes of non-mite species extracted from water mite specimens of *Lebertia quinquemaculosa* and *Lebertia davidcookii* that were preserved immediately after collection, the water mite diets included chironomids, mosquitoes, and numerous oligochaetes [5]. Since these extracts contain DNA from a mixed group of prey organisms, next generation sequencing (NGS) is required to study the diet complexity. NGS analysis revealed that the proportions of chironomids v. oligochaetes in the diets of two species of *Lebertia* varied significantly between the water mite species and also as a function of season [5].

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The present study applies similar NGS methods to study the diets of vernal pond water mites in vernal ponds of Palmer Park, located in Detroit, MI, USA. Palmer Park contains the largest primary urban forest in southeast Michigan. The forest has been relatively well preserved, while the landscape around it has typical urban development, such as pervasive impermeable concrete surfaces, houses, buildings, and monoculture lawns. Palmer Park is located at an average altitude of 190 m (https://mapscaping.com/terrain-map-detroit-michigan/, accessed on 3 January 2026), and near the watershed divide between the Rouge River, Clinton River, and Connor Creek watersheds (https://hiddenwatersblog.wordpress.com/2016/06/14/conner-creek-detroit/, accessed 3 January 2026), approximately 13 km from the nearest major river (Detroit River, altitude 175 m). Its higher altitude in the relatively flat topography of Detroit isolates its aquatic features from fish-containing streams. The park may represent a somewhat isolated ecosystem, including vernal ponds within the forest at Palmer Park. Due to their isolation and their low likelihood of flooding from fish-containing rivers, the vernal ponds in Palmer Park contain a unique and diverse community in which new species of chironomids have recently been described [1].

At Palmer Park, our goal was to study the water mites and their diets in vernal ponds for comparison to previous research conducted in Blue Heron Lagoon [5] and to general observations on water mite diets based mostly on laboratory feeding experiments, reviewed briefly above. We hypothesized that, as in Blue Heron Lagoon, the water mites would be opportunistic generalist predators, not specializing in just one type of prey. We expected that the diets might have a high proportion of mosquitoes, whose adults swarm in great numbers in the vernal pond area, and that other organisms found in the ponds, including chironomids [1], oligochaetes, and ostracods, would also be detected in water mite diets. Although water mites have been shown to parasitize beetles [18–21], this paper provides novel observations indicating that water mites are also predators of beetles.

2. Materials and Methods

2.1. Collection Locations and Sampling Methods

This study began with a survey of organisms (water mites and their potential invertebrate prey) in the vernal ponds of Palmer Park and subsequently applied molecular methods for the study of the diets of the water mites. The study site and collection procedures have been described previously [1]. Briefly, samples were collected from vernal ponds (vernal ponds A, B, and C; see map, Figure 1) in Palmer Park in Detroit, MI by sweeping a 250 μm mesh net near the bottoms of specific spots of the vernal ponds. In the latter part of this study (all of the water mite diet diversity collections in 2024) mites were collected only from Pond A.

The collected sediment and suspended matter, including organisms, were sieved to retain organisms <2 mm and >250 μm [22], a process that retains water mites and an assortment of other invertebrates. The sieved material was poured onto white trays on which individual motile animals "swim out" to the edges of the tray where they were collected in vials and sorted. Invertebrates other than water mites were preserved in 90% ethanol or isopropyl alcohol (rubbing alcohol). Water mites for dietary studies were blanched within one hour of collection in vials immersed in 90 °C water for 60 seconds, which preserves the water mites with their legs extended. Subsequently, ethanol was added to the vials to approximately 70% of the final concentration, as described previously [5]. Preserved animals were stored at 4 °C until analysis. The survey of invertebrates in Ponds A, B, and C took place sporadically over several years (2021 – 2024), with dates given where relevant in the Results. Water mites for dietary studies were collected from February 2024 through June 2024.

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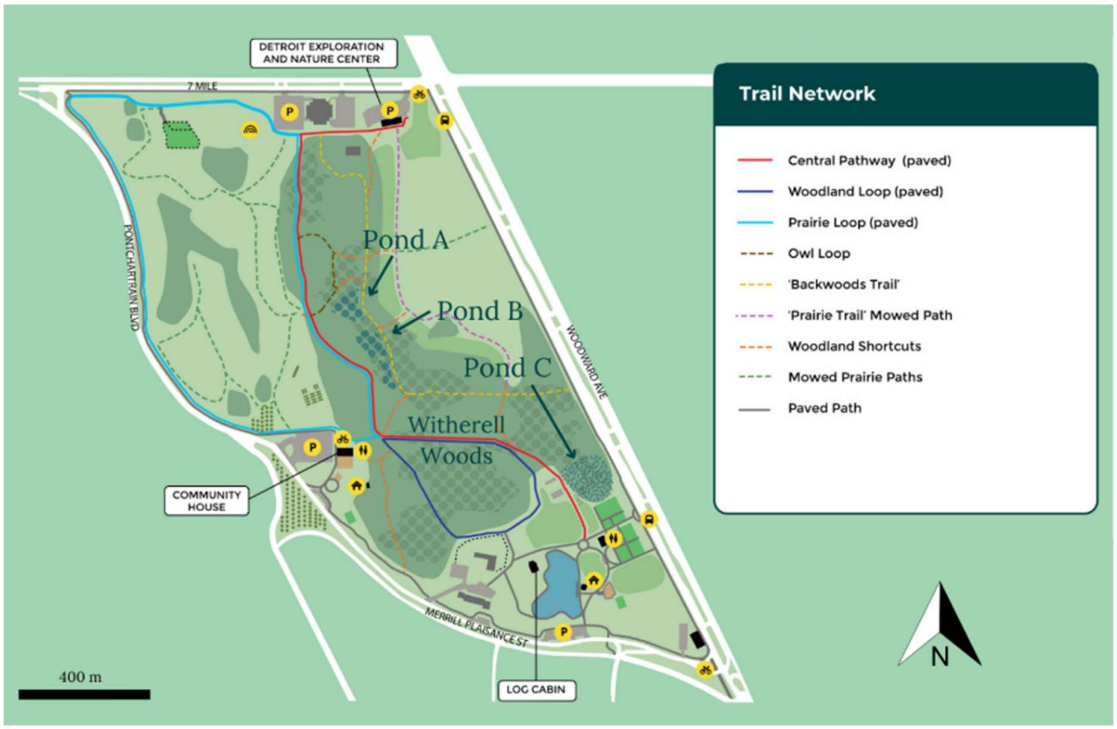

Figure 1. Palmer Park Wayfinding map created by the City of Detroit to be used in the trails of the park. The map outlines the entirety of Palmer Park, and the forest that contains the vernal ponds. Pond A is where all of the data for 2024 was collected, while organisms were also collected from Ponds B and C in previous years. Pond A is at an average elevation of 190 m, and located at 42.42766°N, 83.11741°W.

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Some organisms were also collected overnight in "bottle traps" with a small waterproof rechargeable light inserted for possibly attracting the organisms. This was the method by which most adult diving beetles were collected. Bottle trap organisms were similarly sorted and preserved as the net-caught organisms.

2.2. Microscopy

The dorsal and ventral surfaces of organisms were photographed on a Nikon SMZ 745T (Nikon, Tokyo, Japan) stereo-photomicroscope and associated software during 2021-2023. In 2024, photos of organisms were taken at various focal planes using NIS-Elements D 4.51.00 64-bit, a Nikon Instruments computer program on a Nikon SMZ1270 stereo microscope (Nikon, Tokyo, Japan). Photographs taken at various focal planes were combined to produce an in-depth focused image using Combine ZP (https://github.com/Vincentdecursay/CombineZP, most recently accessed 4 January 2026).

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2.3. DNA extraction and purification

Organisms were then transferred into 1.5 mL centrifuge tubes for DNA extraction and PCR. DNA was extracted by a previously described [23] Qiagen spin column process (https://www.qiagen.com/us/resources/resourcedetail?id=68f29296-5a9f-40fa-8b3d-1c148d0b3030&lang=en, most recently accessed 4 January 2026). After rinsing the outside of the animal extensively with ethanol, whole animals or tissues from larger specimens were individually homogenized with a pestle (cat #1415-5390, USA Scientific Inc, Ocala, FL, USA) in an extraction medium of 180 µL ATL lysis buffer (cat. #19076, Qiagen, Hilden, Germany) in a 1.5 mL polypropylene centrifuge tube. Proteinase K (20 µL of cat. #19133, Qiagen, Hilden, Germany) was added, mixed, and incubated for 3 hours or overnight at 56 °C, after which DNA in the extract was purified with the DNAeasy Blood and Tissue Kit spin column procedure (Qiagen, Hilden, Germany) at room temperature. Final elution from the columns was with Low TE (Invitrogen, Carlsbad, CA) using volumes ranging from 30 – 50 µL, depending on the size of the organism or tissue being extracted. Extracts were frozen at -20 ºC until use.

2.4. PCR Amplification, Amplicon Sequencing, and Bioinformatics Analysis

Next, mitochondrial COI barcodes were amplified using either LCO1490 and HCO2198 [24] or LCO1490 and mLEP [25] as primers, and amplification conditions described by Vasquez et al., (2021) for the study of water mite diets. LCO1490 and HCO2198 are universal primers amplifying COI from almost all animals, including water mites and other invertebrates; mLEP and LCO1490 are designed to amplify COI from most non-Arachnids (i.e., in a mite, amplifying only the DNA not from the water mite host). The LCO1490 and mLEP primers were used together to determine the diet, as they will amplify whatever is in the gut of the mites [5]. The LCO1490 and HCO2198 primers will amplify mainly the dominant DNA sequence from the water mite host or other invertebrate being studied.

PCR products were sequenced by Azenta Life Sciences by either (a) Sanger sequencing in both forward and reverse directions (Genewiz subsidiary, South Plainfield, NJ, USA), using the primers with which the amplicons had been amplified, or (b) Amplicon-EZ Next Generation Sequencing (Genewiz, South Plainfield, NJ, USA). Next Generation Sequencing was used to analyze diet amplicons amplified with the mLep and LCO1490 primers.

Low quality bases (labeled as "N" in the text interpretation) at 5'- and 3'- ends and primer sequences were trimmed, if present, on the 3'- end, and the trimmed sequences were compared to GenBank using BLASTN or MEGABLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome, most recently accessed 4 January 2026). This process returns the family, genus, putative species alignments, query coverage (Q), and percent identity (ID) for GenBank sequences that are most similar to the sample barcodes. In cases of ambiguity (e.g., when forward and reverse sequences have different top matches), an improved consensus sequence for BLAST analysis was obtained by alignment and comparison of chromatogram data using Geneious Prime® 2025.1.2 (GraphPad Software LLC, Boston, MA, USA). In general, we required at least 80% query coverage, accepted percent identities above 90% to reliably identify the genus, and identities above 96.5% to identify the species if the previous GenBank taxon was identified to species level. These threshold values were previously demonstrated to apply among chironomids [17,26]; other threshold levels will be considered in the Discussion. With Sanger sequencing of DNA amplicons from water mites, bioinformatic analyses were almost always able to match host and the predominant diet sequences to a genus, but with high background. Next generation sequencing yielded higher quality multiple sequences that could be matched by BLAST, in some cases to species, and could be compared to COI barcodes of animals in the vernal ponds whose COI barcodes had previously been determined.

In addition, after we determined the preliminary "noisy" water mite host sequences, we designed a set of specific primers, using Primer3 and the preliminary water mite host sequences, to target the water mite host sequences specifically, to verify the valid whole mite sequence with reduced background and improved quality scores. The primers HYD188F (5'-

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TCAGAGCTCCAGATATGGCA-3') and HYD610R (5'-GATCTCCTCCTCCTGCTGG-3') were able to amplify sequences in the Hydryphantidae family, which included Hydryphantes and Parathyas genera that had been preliminarily identified by Sanger sequencing with the Folmer primers.

Sequences obtained through Amplicon EZ sequencing by Azenta Life Sciences were analyzed bioinformatically by a process described by Vasquez et al. (2021). Resultant FASTQ sequence files were clustered with CDHIT with a limit of 3% differences per cluster, and then "operational taxonomic units" were identified by BLASTing the cluster seed sequences against the GenBank database. The goal of the Next Generation Sequencing was to identify the diverse sequences found in the water mites and to determine support for the dominant dietary organisms present in the preliminary high background Sanger sequencing with mLEP and LCO1490 primers.

NGS mite diet sequences obtained in 2024 were also compared to sequences of the invertebrate community organisms that we found in our survey of organisms in the vernal ponds. We compared the Sanger sequences for the invertebrates found in 2021 - 2024 to the Illumina reads for the NGS mite diets. First, we gave the Sanger consensus sequences unique seq IDs, and trimmed the ends if there were >=30% N among 30 nt. Next, we assembled the Illumina R1 and R2 diet reads (previous paragraph), removed bad reads, kept sequences >=200 nt, and converted them into FASTA format. For each Illumina diet sequence sample, we built a database using the unique assembled R1 and R2 reads. Sanger sequences served as query sequences to BLAST against the database of diet sequences of each water mite that had been subjected to NGS sequencing. In Results, we have highlighted bar codes of organisms that matched the diet database sequences with 80% - 90% sequence identities, 90% - 96.5% sequence identities, and >96.5% sequence identities, as in Vasquez et al. (2021).

The best GenBank matches for some of the ostracod sequences gave low "identities" and only to the level of Ostracod Tribe. To improve the taxonomic specificity of their barcodes, several specimens were submitted to EcoAnalysts (Moscow, ID, USA) to obtain a definitive genus identification of their bar code sequences, to be used in searching for ostracod sequences in the water mite diet NGS sequences.

2.5. Biodiversity Analysis

Biodiversity of the organisms in the diets of the water mites was characterized by several biodiversity indices, including the Shannon-Wiener index, Simpson's diversity index, species richness, and species evenness to determine alpha diversity for the water mite diets. Estimates of several of these indices were determined initially using Estimate S [27], on an older computer running a Windows XP operating system. However, after verifying reliability and agreement with Estimate S on preliminary tests, more rapid and easily implemented biodiversity analyses were conducted using the Virtue on-line Biodiversity Calculator (https://www.virtue.gmbl.se/english-content/biodiversity-calculator, accessed 4 January 2026). Neighbor-joining trees illustrating clustering of various COI barcode sequences were created using Geneious Prime® 2025.1.2 (GraphPad Software LLC, Boston, MA, USA)..

2.4. Graphics and Statistics

Graphics (e.g., pie charts), descriptive statistics and statistical comparisons were calculated using Microsoft Excel and GraphPad Prism (version 10.4.1, San Diego, CA, USA).

3. Results

3.1. Survey of vernal pond invertebrates

We conducted a preliminary survey of Ponds A, B, and C in spring and summer 2021, during which we found a variety of mites, midges, and mosquitoes, among other organisms. Similar surveys were conducted during late winter, spring, and summer in 2022, 2023, and 2024. The data here

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provide a qualitative summary of the types of organisms found in the ponds, along with COI barcodes to assist in identification and subsequent comparison to water mite diets.

First, we illustrate the types and numbers of organisms sorted with comparable effort each time on the "swim out" tray on March 22, April 4, and May 3, 2022 (Figure 2). The colored arcs represent the proportions of each type of organism. Although the total number of organisms that were sorted were the same order of magnitude, the proportions changed between the three sets of samples.

Ostracods were prominent on the first two dates but lower in proportion on May 3. Similarly, chironomids were also lower in proportion in May. In contrast, water fleas were not present initially but were identified in great numbers at the beginning of May. The proportion of water mites increased over time. Oligochaetes, copepods and other unidentified organisms were present on all three dates.

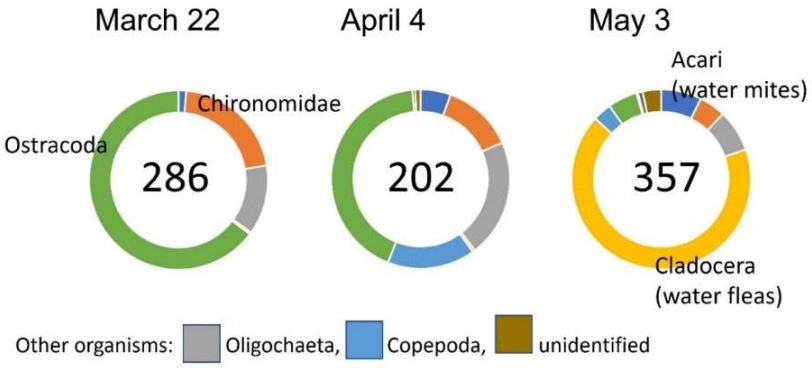

Figure 2. Organisms sorted on "swim out" trays in March – May 2022.

Figure 3 shows a selection of vernal pond invertebrates collected, photographed and barcoded in 2021 – 2024. These include water mites (*Hydryphantes waynensis* and *Parathyas* sp.), oligochaetes (*Lumbriculus variegatus* complex sp. II), copepods (*Acanthocyclops vernalis)*, water fleas (*Daphnia* sp.), ostracods (*Cypris pubera* and *Candona* sp.), flies (*Helophilus fasciatus*), midge larvae (*Chironomus maturus*), beetle larvae (*Acilius* sp. having two distinct COI barcodes), and mosquito larvae (*Ochlerotatus excrucians*, *Culex territans* and *Culex restuans*). We also collected several species of adult diving beetles (*Copelatus glyphicus* and *Hygrotus sayi*) in bottle traps set in the vernal ponds.

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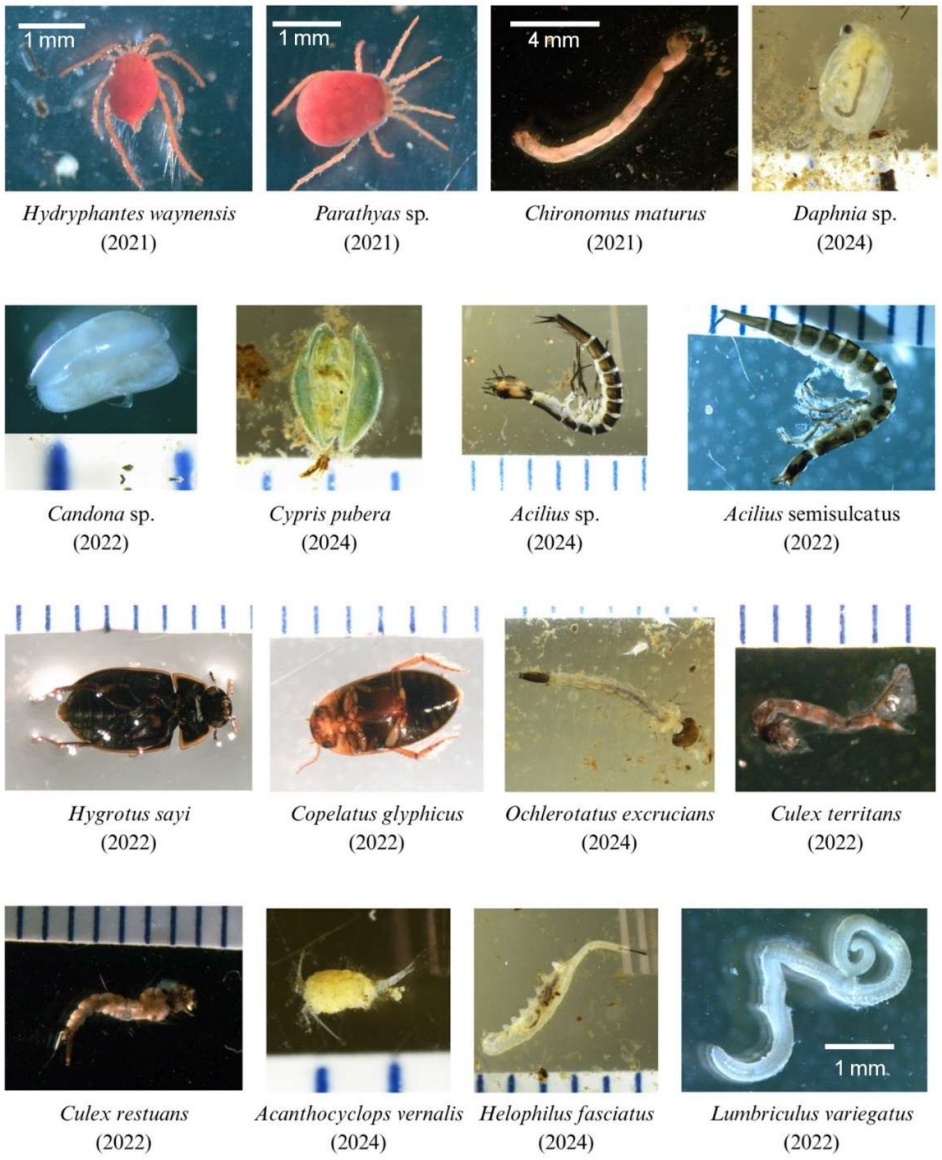

Figure 3. Gallery of representative vernal pond organisms in Palmer Park. Where vertical lines are included in the image, the marks are at 1 mm intervals, for scale. Calibration bars are inserted for scale on other images where ruler marks are not present. Barcodes of these exemplar organisms have been uploaded to GenBank as Accession IDs XXXXX- YYYYY.

The percent identities and best match Accession IDs in GenBank supporting these identifications are summarized in Table 1, which includes the common name, scientific name, percent identity and query match to the top match from a BLAST on GenBank, and the date collected.

One exception to listing the top GenBank match in Table 1 is for ostracods, as a result of additional morphotaxonomy on specimens with a <85% sequence ID in GenBank. Eight ostracods barcoded in 2022 (3 specimens) and 2024 (5 specimens) fell into two clusters: *Cypris pubera* (4 specimens, all >99% ID) and Candonini (tribe; accession ID LC726446.1 was typical; 3 barcodes in 2022, 1 in 2024). The Candonini "matches" in GenBank averaged 83.6% ± 0.2% ID (mean ± S.D.), but their barcodes were 98.9% ± 0.3% identical to each other (Supplement Table S1). Fourteen specimens of ostracods collected on the same dates in 2022 were all identified morphotaxonomically as *Candona* sp. Therefore, we list these barcodes as *Candona* sp. (will be submitted to GenBank as access ID xxxx -xxxxx) upon manuscript acceptance].

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Table 1. Percentage matches of COI sequences to GenBank of the organisms shown in Figure 3.

Common Name	Species identified by GenBank BLAST	%ID[1]	%Q[2]	Date Collected	Best match Accession IDs in GenBank
Water Mite	*Hydryphantes waynensis*	98.1%	86%	05/10/2021	KM101012.1
Water Mite	*Parathyas* sp.	98.6%	98%	05/10/2021	MG317686.1
Non-biting midge	*Chironomus maturus*	99.85%	94%	05/13/2024	MG178863.1
Water flea	*Daphnia* sp.	95.80%	96%	05/13/2024	DQ340827.1
Ostracod	*Candona* sp.[3]	83.41%	92%	02/26/2024	LC726446.1
Ostracod	*Cypris pubera*	99.85%	94%	05/13/2024	MG317588.1
Diving Beetle	*Acilius* sp.	96.49%	96%	04/26/2024	MF637870.1
Diving Beetle	*Acilius semisulcatus*	99.2%	98%	05/04/2022	HM374104.1
Diving Beetle	*Hygrotus sayi*	100%	100%	06/27/2022	KC017092.1
Diving Beetle	*Copelatus glyphicus*	100%	100%	06/27/2022	HQ984351.1
Mosquito	*Ochlerotatus excrucians*	99.4%	99%	03/25/2024	OR891604.1
Mosquito	*Culex territans*	100%	100%	06/27/2022	KR739813.1
Mosquito	*Culex restuans*	99.84%	100%	05/31/2022	GU908085.1
Copepod	*Acanthocyclops vernalis*	99.68%	87%	02/26/2024	MG449045.1
Marsh fly	*Helophilus fasciatus*	97.95%	96%	04/26/2024	MW473969.1
Worm	*Lumbriculus variegatus*	96.61%	97%	03/22/2022	PP139458.1

[1]%ID - the percent identity of the top match from the BLAST against GenBank COI barcodes; [2]%Q – the percent query match of the top match from the BLAST against GenBank COI barcodes; and [3]Identification in GenBank as *Candonini* sp. See text for the more specific identification as *Candona sp*.

Water mites in the vernal ponds are exemplified by the bright red specimens in Figures 3A and 3B, collected in 2021: The species with long swimming setae on its back legs in Figure 3A was identified as *Hydryphantes waynensis* by a 98.2% identity to a previously published barcode (GenBank accession ID KM101012.1). The specimen in Figure 3B lacked the prominent swimming setae and was identified as *Parathyas* sp. with a COI barcode that was 98.9% identical to *Parathyas* sp. GenBank accession ID MG317686.1. Similarly, Figure 4 shows water mites collected from Palmer Park Pond A in 2024.

At least three species of water mites in the family Hydryphantidae were present in the pond in the late winter and spring, as determined from similar appearance to the previously identified specimens from 2021-2023 and by clustering on a neighbor-joining tree (Figure 4C). *Hydryphantes* sp. is clearly on a different branch of the neighbor-joining tree than *H. waynensis*. The identification of three water mite species in Pond A was further supported by sequencing the amplicons obtained using HYD188F and HYD610R primers, designed specifically to target this family of water mites. Specimens in 2024 could only be identified to genus level (90% - 96.5% identity to GenBank) as *Hydryphantes* sp. (n = 6; Figures 4A, B), *Parathyas* sp. (n = 8; Figures 4D, E), and *Hydryphantes waynensis* (n=1). The barcode of the one *Hydryphantes waynensis* specimen found in 2024 was 97.85% identical to GenBank KM101012.1; 99.7% identical to the one specimen of *H. waynensis* found in 2021; and differing from typical *Hydryphantes* sp. in the pond by 11.6%.

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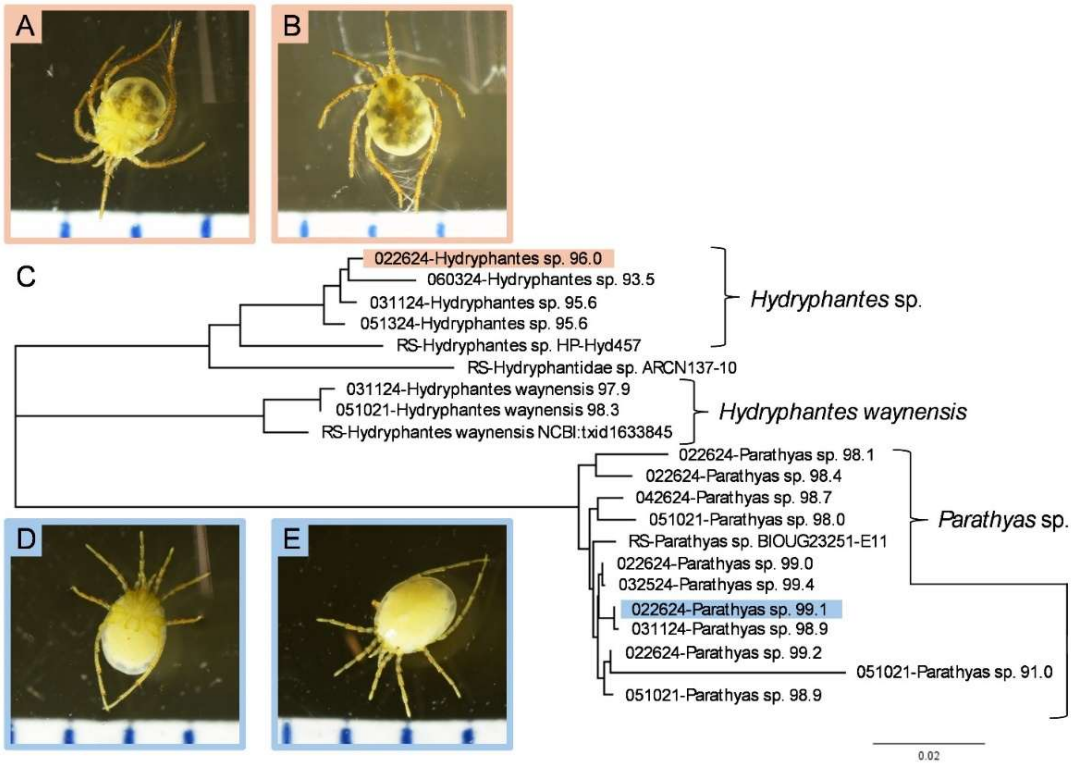

Figure 4. Images and a neighbor-joining tree of COI barcodes of water mites found at Palmer Park. Specimens of (A, B) *Hydryphantes* sp. and (D, E) *Parathyas* sp.: (A, D) ventral side, (B, E) dorsal side. The blue scales mark millimeters. This is not their natural color since they were stored in isopropyl alcohol in which the original color fades. Barcodes of these specimens are highlighted in (C) the neighbor-joining tree. The labels list the collection date, the nearest GenBank taxon match, and the percent identity. The tree includes reference sequences (RS) from GenBank for *Hydryphantidae* sp., *Paratyhas* sp., *Hydryphantes* sp., and *H. waynensis*. GenBank identifies the *Parathyas* and *Hydryphantes* clades only to genus; *H. waynensis* represents a >97% identity to species.

3.2. Molecular Analysis of Water Mite Diets.

3.2.1. Sanger sequencing

Sanger sequencing of mLEP/LCO1490 amplification products gave noisy sequences. A possible dominant component could be identified in five specimens: For three *Parathyas* sp. specimens, the top BLAST matches were *Agabus sp.* (93.43% ID, 95% Q), *Hydroporus niger* (99.29% ID, 90% Q), and *Lumbricus rubellus* (98.23% ID, 93% Q), respectively. *Agabus* sp. and *H. niger* are both diving beetles; *L. rubellus* is an earthworm. For two of the *Hydryphantes* sp. specimens, the top BLAST matches were *Hydroporus* sp. (92.78% ID, 90% Q), and *Dinetus* sp. (93.59% ID, 91% Q), respectively, for each mite. *Dinetus* sp. is a whirligig beetle.

3.2.2. Next generation sequencing (NGS)

However, the full complexity of the diets of these water mites is revealed by NGS analysis of non-mite DNA associated with them (pie charts in Figure 5).

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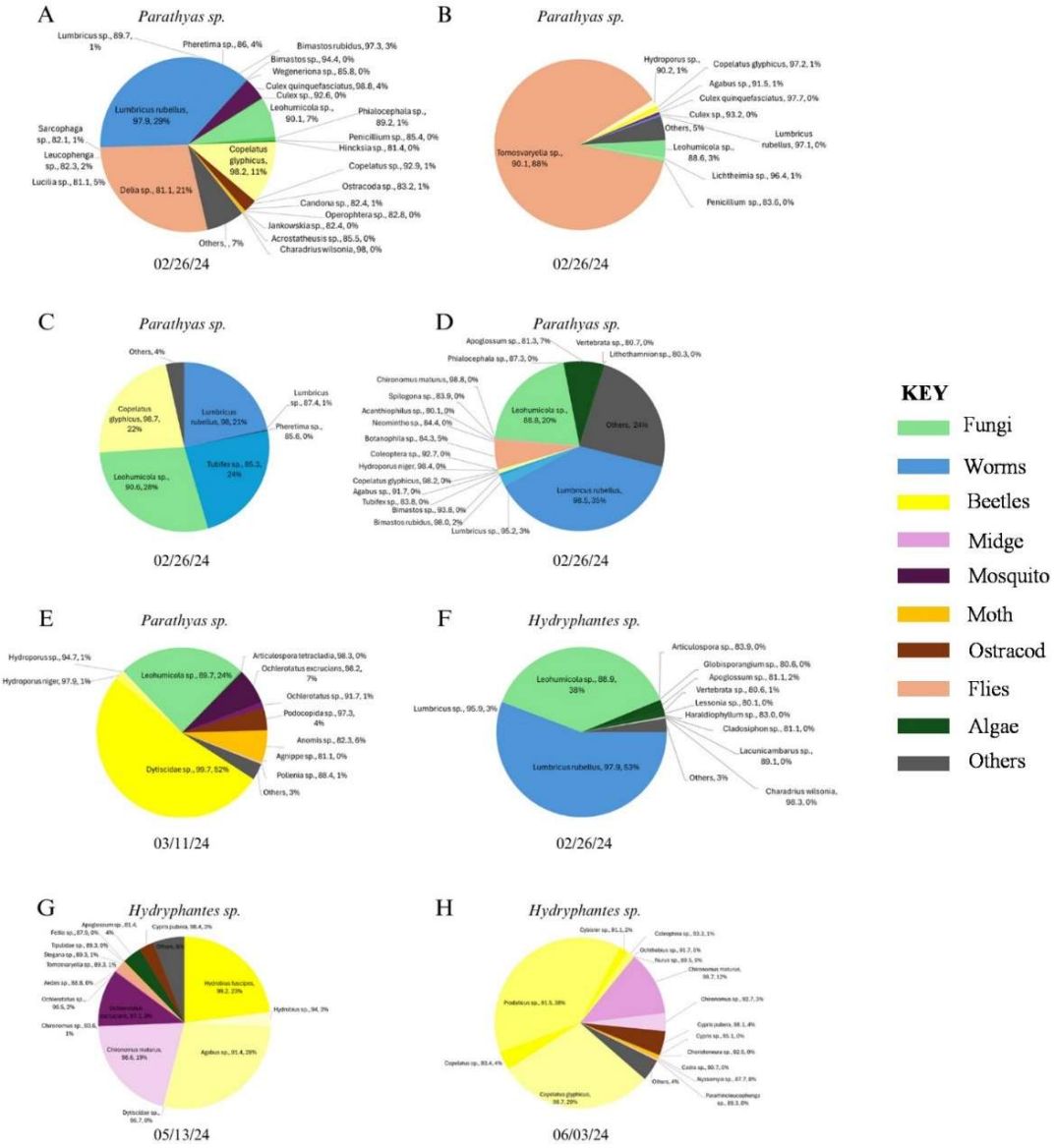

Figure 5. Proportions of various invertebrate barcode sequences amplified in DNA extracts from eight water mites, analyzed with Next Generation Sequencing. The species ID and date collected of each water mite is shown above and below each pie chart, respectively. For each organism, it lists the highest matched genus/species, the percent identity of that match, and the percentage of the whole diet. Some of the slices are combinations of multiple clusters that had the same matched organism, while others are individuals that only appeared once. The combined clusters used a weighted average for the percentage identities, to give the best representation of that diet item.

Pie charts in Figure 5 illustrate the putative dietary components of five *Parathyas* sp. and three *Hydryphantes* sp. specimens, based on the proportions of COI barcodes that matched sequences of specific taxa in GenBank. For *Parathyas* sp. specimens (Figure 5A-E), large proportions of the NGS COI barcodes match GenBank sequences identified as worms (*Lumbricus rubellus*, *Bimastos rubidius*, *Bimastos* sp., and *Tubifex* sp.), beetles (*Copelatus glyphicus*, *Agabus* sp., *Hydroporus niger*, *Hydroporus* sp., and *Dysticidae* sp.), fungi (*Leohumicola* sp.), moths (*Anomis* sp.), and flies (*Delia* sp., *Botanophila* sp., and *Tomosvaryella* sp.). Sequences found in smaller proportions matched GenBank sequences for mosquitos (*Culex quinquefasciatus*, *Culex* sp., *Ochlerotatus excrucians*, and *Ochlerotatus* sp.), ostracods

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(*Ostracoda* sp., *Podocopida* sp., and *Candona* sp.), and algae (*Apoglossum* sp.). *Parathyas* sp. specimens contained very few NGS COI barcodes that matched chironomids.

In the *Hydryphantes* sp. specimens in Figure 5, the largest proportions of their NGS sequences were beetles (*Hydrobius fuscipes, Hydrobius* sp., *Agabus* sp., *Prodaticus* sp., *Copelatus glyphicus,* and *Copelatus* sp.), worms (*Lumbricus rubellus* and *Lumbricus* sp.), fungi (*Leohumicola* sp.), chironomids (*Chironomus maturus* and *Chironomus* sp.), and mosquitoes (*Ochlerotatus excrucians* and *Ochlerotatus* sp.). In smaller proportions, the sequences had best matches to algae (*Apoglossum* sp.), ostracods (*Cypris pubera* and *Cypris* sp.), and flies (*Tomosvaryella* sp. and *Stegana* sp.).

While the above data clearly show great diversity of species associated with these two species of water mites, the percent identity varies among the "best-matched" barcodes and only about half the diet organisms are identified by sequence to species. We illustrate this by example through detailed examination of the diet-associated organisms for the *Hydryphantes* specimen collected on 5/13/2024 in Figure 5G: Organisms that were identified reliably to species by a barcode match of >96.5% (as suggested previously in Vasquez et al. 2023) include *Hydrobius fuscipes*, 99.2 % ID, 23% proportion; *Chironomus maturus*, 98.6% ID, 19% proportion; *Ochlerotatus excrucians*, 97.1% ID, 9% proportion; and *Cypris pubera*, 98.4% ID, 3% proportion. The proportion of the barcodes that were identified to species totaled 54% for this specimen. Considering all of the specimens illustrated in Figure 5, the proportion of diet barcodes that were identified to species ranged from 1% to 54% (median 37% for the five *Parathyas* sp. specimens; median 53% for the three *Hydryphantes* sp. specimens).

Other diet barcodes could be identified only to genus or higher levels, such as family. For example, the match to *Agabus* that accounted for 28% of the barcodes in Figure 5G had only a 91.4% ID match to *Agabus* sp. Figure 5G also had a small proportion (0.3%) of its NGS barcodes identified as Dytiscidae which differed by more than 10% from the *Agasbus* sp. sequences. In Fig. 5E, 52% of the barcodes had a sequence whose top match in GenBank was identified as Dytiscidae sp. (99.7% ID to KR484340.1).

The Dytiscidae sequences from different water mites were analyzed in a neighbor-joining tree and exhibited several well-separated clusters (Figure 6), indicating that several species, from a variety of genera, are included in the Dytiscidae sp. identification. The clusters separate into two major clades, with the upper clade in Figure 6 mostly from the diet of the animal shown in Figure 5G, and with one sequence cluster from the animal shown in Figure 5B. The lower set of sequence clusters were all from the diet of the animal shown in Figure 5E. When sequences of individual beetle specimens (e.g., those listed in Table 1 and NGS-detected sequences of *Agabus* sp., *Hydroporus niger*, *Prodaticus* sp., and *Cybister* sp.) were BLASTed against the various Dytiscidae sp. diet sequences, we found that *Acilius* sp. and *Agabus* sp. sequences had matches >96.5% to the Dytiscidae sequences of the animal illustrated in Figure 5G. In contrast, many of the Dytiscidae sp. sequences in animal 5E matched *Hydroporus niger* sequences, closely enough in some cases to be identified to species, but most having lower identities that suggest a match to at least *Hydroporus* sp. No high percentage ID matches to the Dytiscidae sequences were found for *Copelatus glyphicus*, *Prodaticus* sp., or *Cybister* sp

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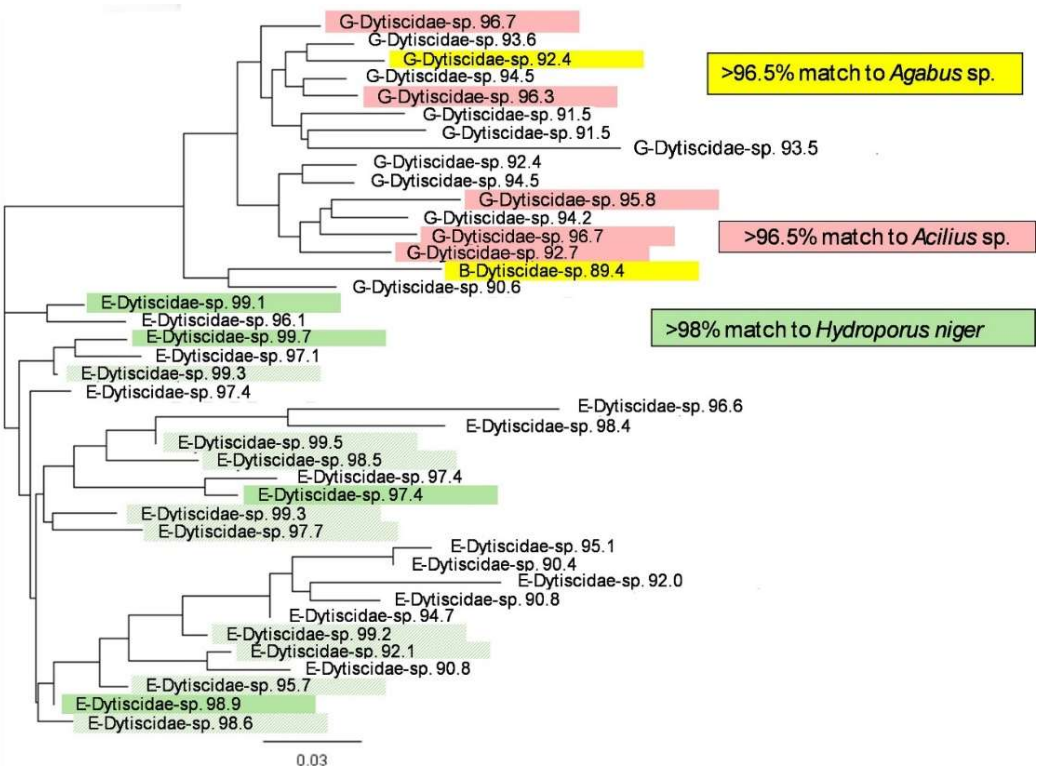

Figure 6. Neighbor-joining tree of the cluster seed sequences from NGS that resulted in Dytiscidae as the top BLAST match in GenBank. Labels identify the animal the sequences were from, referenced to the letter of the pie charts in Figure 5, with the name of the match (Dytiscidae), and the percent identity. Color highlights sequences identified in pair-wise BLASTS that were >96.5% identical to barcodes of animals collected in the pond (*Acilius* sp., pink) or were detected in diet sequences (*Agabus* sp., yellow; and *Hydroporus niger*, green for >98%; lighter (cross-hatched) green for percentage matches of 90% - 98%). The scale bar represents 3% difference in sequence.

Similarly, BLAST was used to match sequences of other organisms that we had collected in the vernal ponds in 2021-2024 to the 2024 NGS diet sequences (Table 2). For the analysis, any NGS sequences to which the non-mite organisms had <90% sequence identity were not counted as an identified part of the mite diet. Starting with the beetles found in the pond, the results show that all the water mites (A-H) had *Copelatus glyphicus* sequences, as evidenced by sequence matches in all specimens of >98%. *Acilius* beetle DNA was found with >90% identity in all water mites but F, indicating that this genus of beetles was available to the water mites; however, the % identities enable us to identify *Acilius* in the diets only to genus level. Since none of the % identities for *Hygrotus sayi* and *Hydrochara obtusata* were above 90%, we can't identify these genera with confidence as part of the 2024 mite diets. For the three different species of mosquitoes found in the pond, all the mites except for A and F had diet identities >99% for *Ochlerotatus excrucians*. *Culex* species (*C. territans* and *C. restuans*) had matches of 90% - 95% identity in water mites A, B, F, G, and H. The diet sequences of every mite except F had matches to *Chironomus maturus* above 99% and to the ostracod *Cypris pubera*, above 98%. All the mites, except mite A and F, had sequences from the ostracod, *Candona* sp. All the mites except mite B and F, had marsh fly sequences. Only mite A had the copepod, *Acanthocyclops vernalis,* in its diet, with a 99.4% sequence match. None of the mites had high percentage matches to water fleas, *Daphnia* sp., the copepod, *Eucyclops cf. estherae,* and the worm, *Lumbriculus variegatus.*

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Table 2. Percentage matches of COI sequences to GenBank of the organisms shown in Figure 3.

Common Name	Species identified as intact organisms in Vernal Pond A	% ID	% Q	% ID match to NGS diet item (letters A – H correspond to individual mite specimens analyzed in Figures 5A-5H)							
				A	B	C	D	E	F	G	H
Non-biting midge	*Chironomus maturus*	99.85%	94%	99.4	99.4	99.4	99.4	99.4	81.5	99.4	99.7
Water flea	*Daphnia* sp.	94.25%	95%	None	None	None	None	None	None	None	None
Ostracod	*Candona* sp.*	83.41%	92%	None	97.6	98.5	97.9	98.5	80.3	97.3	97.0
Ostracod	*Cypris pubera*	99.85%	94%	98.5	98.8	99.1	98.8	99.1	83.4	99.4	99.4
Diving Beetle	*Acilius* sp.	96.49%	96%	91.1	98.5	91.1	94.5	99.4	85.9	99.4	99.4
Diving Beetle	*Acilius semisulcatus*	99.2%	98%	88.4	94.0	89.0	89.9	94.6	80.2	94.9	94.6
Diving Beetle	*Hygrotus sayi*	100%	100%	88.5	87.9	89.4	88.7	88.4	86.5	89.0	89.4
Diving Beetle	*Copelatus glyphicus*	100%	100%	99.0	99.0	99.0	99.0	99.0	98.4	99.0	99.4
Aquatic Scavenger Beetle	*Hydrochara obtusata*	100%	100%	88.0	88.0	88.3	88.6	88.3	85.1	89.3	89.9
Mosquito	*Ochlerotatus excrucians*	99.4%	99%	87.4	99.4	99.4	99.7	100.0	87.1	99.7	99.7
Mosquito	*Culex territans*	100%	100%	90.9	90.2	86.4	86.4	87.0	None	88.0	87.3
Mosquito	*Culex restuans*	99.84%	100%	94.0	93.1	88.9	89.0	88.3	93.6	94.6	93.3
Copepod	*Acanthocyclops vernalis*	99.68%	87%	99.4	84.8	None	None	None	None	None	None
Copepod	*Eucyclops cf. estherae*	99.53%	98%	None	None	None	None	80.5	None	None	None
Marsh fly	*Helophilus fasciatus*	97.95%	96%	90.7	88.6	90.7	90.4	90.4	87.0	90.4	91.4
Worm	*Lumbriculus variaegatus*	96.61%	97%	None	None	80.7	None	None	None	None	None

* *Candona* sp. was originally identified in GenBank as tribe *Candonini* sp.; however, our morphotaxonomic analysis indicated that these specimens were *Candona* sp. and their sequences were >99% identical to sequences found in NGS diet analysis.

3.2. Diet Diversity

Since the above data indicated that both species of water mites had diverse generalist or opportunistic diets, as compared to animals specializing in only one prey type (e.g., [28,29]), we sought to compare and contrast the diet diversity of these water mites to each other and to previous studies of water mites [5]. Diversity metrics were calculated for each specimen's diet (Table 3). Animals A – H correspond to the animals analyzed in Figures 5A – 5H. The taxonomic richness of the diets varied from as many as 25 taxa in animal A (*Parathyas* sp.) to as few as 7 in animal C (also *Parathyas* sp.). The diet species richness of both *Parathyas* sp. and *Hydryphantes* sp. averaged 15. The Shannon-Weiner index for the diets averaged around 1.5 for both *Parathyas* sp. and *Hydryphantes* sp., which means there was almost no difference between the genera. The average Simpson's diversity index for the diets of both genera of water mites had values of approximately 0.3. Simpson's diversity index yields values between 0 (no diversity) and 1 (maximal diversity), indicating that the water mite diet diversity is on the lower end of this diversity scale. The evenness values averaged 0.58 for both genera, as well, which is marginally closer to 1 in the range between 0 and 1, indicating that taxa in the diets are somewhat similar in abundance across various prey. We attempted to compare the beta diversity between the diets in the *Parathyas* specimens and the *Hydryphantes* specimens, but the numbers of animals were too small to draw statistically reliable conclusions. Furthermore, on PCoA plots (data not shown) the points are widely dispersed and overlap between the two species.

Table 3. Diet diversity statistics of *Parathyas* and *Hydryphantes*[1].

Animal ID[2]	Richness S	Shannon H	Simpson's D	Evenness E
P-5A	25	2.248	0.157	0.698
P-5B	11	0.58	0.777	0.242
P-5C	7	1.523	0.233	0.783
P-5D	20	1.73	0.234	0.578
P-5E	12	1.462	0.339	0.588

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H-5F	13	1.103	0.423	0.43
H-5G	16	1.979	0.183	0.714
H-5H	16	1.706	0.255	0.615
Parathyas average	15	1.51	0.348	0.578
Parathyas SD	7.3	0.6	0.2	0.2
Hydryphantes average	15	1.60	0.287	0.586
Hydryphantes SD	1.7	0.4	0.1	0.1

[1]Diversity indexes were calculated with Virtue (https://www.virtue.gmbl.se/english-content/biodiversity-calculator , accessed 4 January 2026)

[2]Animal ID is coded as follows: X-5Y, where X indicates *Parathyas* sp. (P) or *Hydryphantes* sp. (H) and Y indicates which specimen corresponding to Figures 5A – 5H was evaluated.

Discussion

These data on water mites and their diets deepen our understanding of the roles of water mites in a vernal pond food web. In vernal pond A in Palmer Park, we identified at least three different species of water mites, which prey on a diverse assortment of organisms. Generally, we have used differences in COI barcodes greater than 3.5% as indicative of different species, so the distinct branches on the neighbor-joining tree (Figure 4) and the average distance of *Hydryphantes* sp. from *H. waynensis* of 11.6% is good evidence that *Hydryphantes* sp. is a species of water mite that is distinct from *H. waynensis*. Intraspecific pairwise differences of COI barcodes among Acari are not well-studied and may be complicated by cryptic or "species complex" ambiguities [30]; however, in the absence of contradictory evidence, assuming specimens of water mites that differ by more than 3.5% are different species seems reasonable and pragmatic. Among previous studies of water mite species in vernal water bodies, a study in Poland reported various species of *Hydryphantes*, *Thyas*, *Piersigia*, *Piona*, and [31]. A study of water mites in vernal ponds in Ohio found specimens of *Hydryphantes waynensis* [32]. Temporary ponds in Siberia had a wide range of water mite species that included several species of *Parathyas*, as well as *Limnesia* and various *Pionidae* [33]; other vernal ponds in Siberia reportedly had various species of *Hydryphantes* [34].

The invertebrate prey of water mites in the vernal ponds include multiple species of worms, beetles, mosquitoes, flies, moths, ostracods, and chironomids (Figure 5 and Table 2). A previous study on unidentified water mites in vernal ponds in eastern Washington state speculated that they might prey upon the ostracods that were common in the ponds [35]. In comparison to previous research on water mites at Blue Heron Lagoon [5,22], various dietary similarities and differences are present. First, the assemblage of water mites at Blue Heron Lagoon is very diverse with large numbers of *Lebertia* sp., *Arrenurus* sp., *Neumania* sp., and also smaller numbers of approximately 13 other genera [22], while at Palmer Park we found only *Hydryphantes* sp. and *Parathyas* sp. Second, the Palmer Park mites have significantly fewer chironomids and water fleas in their diets. This could be due to the small size of vernal pond A compared to Blue Heron Lagoon and the fact that we found only one species of chironomid larvae (*Chironomus maturus*) in the pond in 2024. However, adult chironomids captured by nets in the vegetation around the ponds included several other unique species of chironomids (Namayandeh et al., 2024). In earlier years (2021-2023), chironomids with different sequence barcodes were seen (data not shown), but they were not present with high percentage matches in this dietary study of 2024. *Daphnia* was found in the diets of some water mites in Blue Heron Lagoon but were not detected in the 8 animals examined from the Palmer Park vernal pond. Perhaps the biggest difference was that beetle sequences were observed in the diets of the water mites in Palmer Park and not at all in the water mites from Blue Heron Lagoon [5].

These differences in community sizes and diversity may be attributed in part to the temporal variation between the vernal ponds and the lagoon. The lagoon exists all year round, even if it freezes over, while the vernal pond is temporary and is dried up for parts of the year. Other natural differences are that the vernal pond is in a forested area and is filled with decaying fallen leaves; whereas, the Blue Heron Lagoon has excellent water quality (it is adjacent to one of the water intakes for Detroit's drinking water) and is flushed through by exchanges with the waters of the Detroit

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River. These and other environmental factors such as the absence of fish in vernal ponds may underlie the lower frequency of chironomids and water fleas in the diets of *Hydryphantes* sp. and *Parathyas* sp., compared to the diets of *Lebertia* sp. at Blue Heron Lagoon. Numerous other studies of water mites have indicated that "typical" water mite diets include chironomids, cladocerans, copepods, and ostracods [12–14,16,36]; however, none of those previous publications have recognized beetles as part of water mite diets.

Beetles were a significant proportion of the diets of the water mites at Palmer Park. Finding beetles in the diet of water mites is a novel discovery for which we could find no previous study. The water mites contained DNA from a wide variety of beetles, including *Copelatus glyphicus*, *Copelatus* sp., *Dytiscidae* sp., *Hydrobius fuscipes*, *Hydrobius* sp., *Hydroporus niger*, *Agabus* sp., *Acilius* sp., and *Prodaticus* sp. These beetle species and genus identifications were based on sequence CDHIT clustering seeds that matched GenBank accessions within 3%. A study of a large number of beetle species and genera indicated that pairwise differences of intraspecific barcodes are generally less than 2% and that interspecific differences within same genus are >4% [37], although species delimitations among beetle species barcodes have been suggested to be as high as 9% [38]. Therefore, these species and genus identifications are well within the distances suggested for reliable identification of beetle species.

Beetles are well known as inhabitants of vernal ponds [39,40]. Dytiscidae is the family for the genera *Copelatus*, *Hydroporus*, *Agabus*, *Acilius*, and *Prodaticus* (subgenus of *Hydaticus*) of aquatic beetles that were found in the vernal ponds at Palmer Park. All Dytiscidae beetles undergo a complete aquatic metamorphosis, where they have an egg, larvae, pupa, and adult stage [19]. *Copelatus glyphicus* has overwintering adults that typically inhabit temporary, lentic, aquatic habitats, and they deposit their eggs there or in adjacent flooded aquatic habitats in the summer [41,42]. *Hydroporus niger* and *Acilius* sp. lay their eggs in the spring in ponds; most adults emerge in June, and the adults overwinter in ponds or surrounding terrestrial environments [43,44]. *Agabus* sp., similar to most Dytiscidae beetles, produces one generation of offspring per year, and different species have distinct life cycles regarding where, terrestrial or aquatic, and when, adult or egg, overwintering occurs [45]. *Hydaticus* sp. (subgenus *Prodaticus*) also overwinters as adults, but in terrestrial habitats, specifically in leaf litter in European species [44]. *Hydrobius* sp., and *Hydrobius fuscipes*, the only beetles we found in the mite diets that were part of a different family, Hydrophilidae, have very similar characteristics to the Dytiscidae beetles. The adults overwinter, their populations peak in spring, and they reproduce once a year [46]. It was uniquely mentioned that they do well in ephemeral ponds in the spring due to the peak abundance of leaf litter, which provides more food and shelter [46]. This can most likely explain the occurrence of the diverse amount of beetle species present in the vernal ponds in Palmer Park, especially since it is wettest during the spring and early summer. The diversity and aquatic developmental stages of all of these beetle species present in the pond provided the water mites with a variety and abundance of prey.

Despite the above large differences in the water mite diets at each location, the studies at Palmer Park corroborate the novel finding by Vasquez et al. (2021) that oligochaetes are an important component in the diets of water mites [5]. Vasquez et al. (2021) showed a high proportion of oligochaetes in the diets of *Lebertia quinquemaculosa*, less so in the diets of *L. davidcooki*. Both *Parathyas* sp. and *Hydryphantes* sp. specimens (overall, half of the eight animals examined) exhibited large proportions of worms in their diets (median = 40%, when present in the diet); however, the numbers of animals for which worms were observed in the diets is too small to make a statistical statement about whether worms were more commonly ingested by *Hydryphantes* sp. or not.

Using the data we have gathered on the diets of *Hydryphantes* sp, and *Parathyas* sp., we can describe them as opportunistic carnivores. The diets illustrated in Figure 5 and the diet diversity indexes in Table 3 show that these vernal pond water mites consistently ate a wide variety of organisms and did not specialize on only one type of prey or group of organisms, which might be a key strategy for optimal foraging in water mites [47], especially in the face of ephemeral conditions [48]. The evenness estimate is in the mid-range for this variable. Although individual animals may

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eat more of certain organisms, such as flies, beetles, and worms, this likely can be attributed to greater availability, size of the prey, and higher nutritional values compared to less consumed prey. Further evidence to support the opportunistic nature of mite predation is that almost all the types of species found while sampling (chironomids, worms, mosquitoes, ostracods, water fleas, etc.), were also found in their diets (Table 2).

The NGS data on water mite diets exhibited greater biodiversity than was found through manual sampling of the pond. Thus, Figure 5 (diet diversity) shows a greater richness of unique taxa and species in water mite diets, compared to the diverse specimens caught by us and illustrated in Figure 3 and Table 2. The NGS data had several unique sequences of beetles, worms, mosquitoes, fungi, algae, flies, and moths, compared to the barcode sequences of invertebrates in the pond. This supports our hypothesis that water mite predation can reveal a "hidden biodiversity" within aquatic ecosystems, as they eat specimens that we are unable to collect through traditional sampling techniques. Water mites can be useful tools in assessing and characterizing the biodiversity of aquatic habitats.

We are uncertain whether the presence of a fungus (Figure 5) in several specimens might indicate it was part of the diet or a possible parasite or infection. Water mites are not typically thought to consume algae and fungi [4]. Explanations for the presence of algae and fungi in these extracts include the possibility that algae and fungi could have adhered to the exoskeletons of the water mites despite efforts described in the Methods to clean the specimens prior to extraction of the DNA. Another potential explanation for the presence of fungal and algal sequences could be that water mites had ingested organisms that themselves had consumed fungi or algae. Future experiments may examine these possibilities by analyzing only extracts from the internal soft tissues of the water mites and by doing similar studies of the diets of the water mites' prey (e.g., examining the diets of diving beetles using similar techniques). Regardless of the inclusion of the fungi and algae in the results, the diversity of the diet is significant.

The sequences of organisms in the diets of water mites differed in many cases from species that we found as whole animals in our samples, but they are from among the same groups of species. Furthermore, we did not have a large enough sample size nor collections over several years to determine if there was a seasonal variation in available prey or dietary patterns. Very likely, a seasonal variation does exist since the diversity and numbers of prey increase from late winter to mid-summer (Figure 2) due to the natural, seasonal rise in temperature and the large influx of water to the ponds as snow melts and spring rains occur, which allows for more productive ecosystems.

Future directions for this research include species-level identification of the water mites and their prey. Using reference barcodes in GenBank can take taxa identification only as far as the reference sequences are closely matched (i.e., at least 96.5% identical) to the database sequence and only if those prior sequences had themselves been identified to species level. In this study only *Hydryphantes waynensis* met this criterion. For future collections, we will collaborate with morphotaxonomic experts to identify the exact species *Hydryphantes* sp. and *Parathyas* sp. or to determine if they are novel and could be assigned a new species name. Since the process that we used to extract from the mites in the present study destroyed the structure of the source animals, morphotaxonomic work must wait for future studies and adoption of methods that extract the gut DNA while leaving the exoskeleton intact for morphotaxonomic species identification. Similarly, several of the prey species sequences, such as the Ostracods, could only be determined to the genus-level; collaboration with an ostracod expert may enable more specific identification of these water mite prey. Previous comparative observations of the diets of two species of *Lebertia* sp. suggested that water mite species with a more elaborate "web" of swimming setae consumed a significantly higher proportion of chironomids in their diet [17]. Between *Hydryphantes* and *Parathyas*, we observed the same trend—*Hydryphantes* had the more elaborate swimming setae and also a higher proportion of chironomids in their diet. To determine if this trend is statistically and biologically significant will require the diets of more specimens of water mites to be determined and direct observations of feeding behavior; this will be one of the objectives for future field seasons. Finally, a future objective

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of this research will be to determine the life stage and species (in cases where barcodes reveal only the genus) of aquatic beetles that these water mites are ingesting. While water mites are known to consume insect eggs [4,49–51] and 7 genera of water mites were listed as having "insect eggs" or "dipteran eggs" as prey [4], beetle eggs have not been studied in these previous observations, nor have predation on other stages of aquatic beetle life cycle been investigated. Future studies should determine the life stage at which beetles are preyed upon and whether water mite predation significantly affects beetle populations and their roles in the ecology of vernal ponds. Future research should also explore further the associations with fungi and algae, to determine if these are part of their diets, an artefact of adherence of these organisms to the water mite exoskeletons, an infection of the water mites, or a reflection of DNA in the diets of the organisms they ingested.

4. Conclusion

This paper expands the knowledge of water mites and their diets. In Palmer Park, at least three species of water mites were found in the vernal ponds, *Hydryphantes* sp., *Parathyas* sp., and *Hydryphantes waynensis*. Diet diversity analyses indicated that *Parathyas* sp. and *Hydryphantes* sp. ingested DNA from similar organisms, including a high proportion of worms and beetles. Water mite diets may also prove to be a useful tool for assessing vernal pond biodiversity. Indeed, this paper reports the novel discovery of substantial amounts of beetle DNA, from *Copelatus glyphicus* and at least 10 other beetle taxa, associated with every specimen of water mites. The presence of significant amounts of beetle and worm DNA in water mites further supports the idea that water mites are opportunistic carnivores.

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