



Water mite assemblages reveal diverse genera, novel DNA barcodes and transitional periods of intermediate disturbance

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Abstract

Water mites are important constituents of aquatic ecosystems, but their biodiversity is poorly understood. The goal of this study was to improve knowledge of water mite assemblages in the Detroit River through combined use of morphological and cytochrome oxidase I (COI) DNA barcode data and to elucidate seasonal water mite diversity. The diversity of water mites collected from Blue Heron Lagoon at Belle Isle, an island in the Detroit River, is described. Novel DNA barcodes for *Albia*, *Hydrochoreutes*, *Madawaska*, and *Axonopsis* are reported with a species level barcode for *Lebertia*. Novel DNA barcodes may represent the presence of previously undescribed variants or new species of several genera. The prevalence of water mites is higher in the summer, but a different pattern is observed for diversity. The diversity of water mites, by several measures, varies seasonally with lower diversity in summer and winter months and higher diversity during seasonal transitions. For these organisms, we interpret seasonal change as an intermediate disturbance resulting in increased biodiversity.

Keywords Biodiversity · Blue Heron Lagoon · Cytochrome oxidase 1 (COI) · Belle Isle · Detroit River · Intermediate disturbance

Introduction

Water mites are predatory, and often parasitic, arachnids that inhabit aquatic habitats worldwide. Although water mites are among the most biologically diverse microinvertebrates, with over 6000 species described, only about half have been described in North

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America (Di Sabatino et al. 2008). With diverse chemical sensitivities that make them useful as bio-indicator species in environmental studies (Goldschmidt et al. 2016; Klemm et al. 1990; Miccoli et al. 2013), water mites have also been suggested as potential agents for biocontrol of pests such as mosquitos (Atwa et al. 2017; Di Sabatino et al. 2000; Goldschmidt et al. 2016; Werblow et al. 2015). Despite their great diversity and potential utility, not much is known about the genetic diversity of water mites in North America. Although some groups have started to include DNA barcodes in their descriptions of water mites (Fisher et al. 2015; Pesic and Smit 2014), many taxa lack representation in public sequence databases. For example, none of the 16 water mite species known from the Great Lakes are represented in the Barcode of Life DataSystem (BOLD) (Trebitz et al. 2015).

The present study investigated water mite diversity in Blue Heron Lagoon, a lagoon connected to the Detroit River at Belle Isle, Detroit, MI, USA. Belle Isle is a 400 hectare island located in the Detroit River, which connects Lake St. Clair (upstream) to Lake Erie (downstream; Fig. 1a). The Detroit River is an EPA designated Area of Concern (AOC) (Great Lakes Area of Concern Detroit River 1987) due to its close proximity to an urban center with overflowing sewers, industrial discharges, high levels of polychlorinated biphenyls (PCBs), bacterial contamination, loss of fish and wildlife habitat, and degradation of fish and wildlife populations (Great Lakes Area of Concern Detroit River 1987). The Blue Heron Lagoon/Detroit River Restoration Project funded by the Environmental Protection Agency (EPA) recently connected the Blue Heron Lagoon to the Detroit River in order to improve these habitats (EPA GL—00E00474-2). Frequent assessment of the diversity of organisms in an ecosystem is necessary to evaluate the health of sensitive restored habitats like the Blue Heron Lagoon. Assessing diversity and persistence of bioindicator aquatic invertebrates, such as water mites, is important but to our knowledge there has never been a study of water mite taxa from the Detroit River.

The goal of this study was to use both DNA barcode and morphological data to study the organismal diversity of water mites in Blue Heron Lagoon. To explain the changes in water mite community assemblages that we observed, we propose a novel application of the Intermediate Disturbance Hypothesis (IDH). The IDH model tracks changes in the organismal diversity of a community with respect to the intensity and frequency of ecological (i.e. abiotic and biotic) disturbances (Connell 1978). The IDH model has been used to characterize ecological disturbance regimes spanning both spatial and temporal phenomena (Wilkinson 1999).

Since those early studies others have studied this model in aquatic systems including phytoplankton studies (Liu et al. 2019). However, more research is needed to understand how seasonal disturbance might play an important role in aquatic diversity in light of current anthropogenic effects on climate (Weber et al. 2013). As will be described in this paper, water mite assemblages revealed transitory periods of higher water mite diversity that to our knowledge represent the first field observation of this phenomenon for water mites. Additionally, DNA barcodes revealed the presence of at least 17 genera of water mites with potentially multiple species represented among several genera.

Materials and methods

Water mite sampling

Water mites were collected from benthic sites using 250 μm circular mesh nets at depths of 0.5–1 m along the shore of Blue Heron Lagoon (Fig. 1). Water mites were collected using the method described in Smith et al. (2010). Briefly, after collecting at appropriate depth with agitation of the substrate and passing through emergent vegetation, samples were sieved through a 2 mm sieve to remove coarse, larger material, and water mites and other benthic organisms were captured on a 250 μm sieve. One sample was composed of 10 swoops of the circular net through the suspended substrate and vegetation. Five samples were collected on each sampling day and transferred back to the laboratory in a plastic container. The samples were poured into a sorting plate, and live mites, which are easily distinguished by their size, shape, and swimming behavior, were pipetted into holding vials. Mites were then “blanched” by submerging the vial holding the mites in 90 °C water for one minute, which euthanizes the mites with their appendages extended (Ray Fisher and Ashley Dowling, personal communication). This blanching method is important to assist with proper identification since it allows the ventral region to be easily analyzed. Mites were then preserved in 95% ethanol. Water mite sampling was done on the following dates:

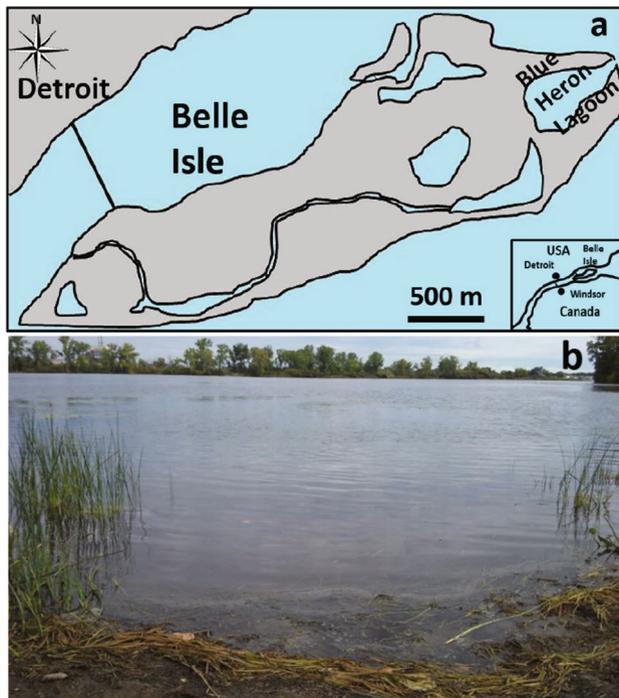


Fig. 1 Water mite collection site. **a** Blue Heron Lagoon location on Belle Isle, which is situated in the Detroit River, on the American side of the river between the US and Canada (inset). **b** Typical collecting site along the shore of Blue Heron Lagoon. Collecting occurred just beyond the rooted vegetation growing along the shore

July 22nd 2016, August 8th 2016, October 15th 2016, November 11th 2016, February 23rd 2017, April 19th 2017, and June 5th 2017. Water mites were also collected on other days when particular genera were the target of the collection.

Water mite identification

Water mites were identified using a two-tier method involving morphological and genetic analysis. Morphological identification primarily used published keys by Smith et al. (2010) and Cook (1974) with additional keys used for specific genera such as the key for *Lebertia quinquemaculosa* (Marshall 1929) and the recently updated key for *Madawaska* (Smith et al. 2015). Water mites that were too damaged for proper identification or that were immature were listed as unidentified mites. The authors also consulted expert water mite taxonomists, including David Cook (retired; Phoenix, AZ, USA; emeritus professor Wayne State University), Ian M. Smith (retired; Ottawa, Ontario, Canada; Canadian National Collection of Insects curator) and John Pfeiffer (active; Moscow, ID, USA; freshwater taxonomist). For morphological documentation, dorsal and ventral aspects of representative water mite specimens were photographed with a SPOT camera mounted on a Nikon SMZ stereomicroscope.

Molecular genetic analysis

For genetic analysis, DNA was extracted from representative water mites from each genus using the Qiagen Easy tissue extraction protocol as in Vasquez et al. (2016). Briefly, mites were incubated in proteinase K enzyme (Qiagen, Cat. #19131) for 3 h or overnight at 57 °C. When necessary, DNA was extracted by puncturing water mites with sharp minutien pins to allow water mite lysate to ooze out and a voucher of the exoskeleton to be retained for subsequent morphological analysis and archival storage. The barcode region of the COI gene in the extracted mite DNA was amplified using the Folmer primers, HCO2198 and LCO1490 (Folmer et al. 1994; Hebert et al. 2003), and PCR products were submitted to GENEWIZ (Plainfield, NJ, USA) for bidirectional sequencing, as previously described by Vasquez et al. (2016).

Bioinformatics

Forward and reverse sequences were aligned and quality-checked using DNA Baser software (Heracle BioSoft, Pitesti, Romania) as previously described (Vasquez et al. 2016). Sequences that passed the quality check were curated so that, when possible, at least one to two representative sequences from each water mite genus were included in our analysis. Neighbor joining trees were constructed using a curated subset of sequences in MEGA6 (Tamura et al. 2013). All sequences were compared to GenBank using BLAST (Altschul et al. 1990; Benson et al. 2007; Ratnasingham and Hebert 2007) and the presence of sequences in the Barcode of Life Database (Altschul et al. 1990; Benson et al. 2007; Ratnasingham and Hebert 2007) was checked using the BOLD systems identification request portal (https://v3.boldsystems.org/index.php/IDS_OpenIdEngine). These sequence data have been submitted to the GenBank database under accession numbers: MG773261 to MG773265, MG835758 to MG835761 and MG811658 to MG811687.

Diversity analysis

Abundance, diversity, richness, and similarity of mite assemblages collected during each of the approximately bimonthly collections were investigated by methods identical to those described previously by (Dorsey et al. 2013; Gotelli and Colwell 2001; Vides-Hernandez et al. 2017). Briefly, we calculated proportional species abundance (p_i) values in each collection, plotted them in rank abundance curves to examine richness and evenness, and generated a Euclidean distance tree diagram using Statistica 6.0 (StatSoft, <http://www.statsoft.com>) to determine the presence of a temporal tree clustering pattern. The resultant clusters of collection events were then subjected to analysis of their α -diversity using the Shannon Diversity Index (H) which has been used in previous studies of water mite generic and family diversity (Goldschmidt et al. 2016; Gonzalez et al. 2018; Miccoli et al. 2013), adjusted for different numbers of individuals in each collection using a rarefaction subsampling technique (Sanders 1968) repeated for a total of 100 iterations. Beta diversity calculations examined similarity indices (Jost et al. 2011) of the temporal clusters using the Sorensen method used in other water mite generic diversity studies (Di Sabatino et al. 2008) as well as the Morisita–Horn Index, which is relatively robust with respect to the abundance of rare species (Jost et al. 2011). The alpha- and beta-diversity calculations were computed with the program EstimateS v9 (Colwell 2013) using data triplets comprising the sampling month, genus identification, and relative abundance values for each of the temporal mite assemblages identified by the cluster analysis.

Results

Micrographs of the water mites of Blue Heron Lagoon

Representative mites from all population types present in Blue Heron Lagoon were photographed before molecular analysis. The panels in Fig. 2 are all paired micrographs of the dorsal followed by the ventral view of the mite. Panels A and B represent the deuteronymph form of *Arrenurus* the most common mites in our collection with panels M and N illustrating the adult form of *Arrenurus*. *Oxus* is represented in panels C and D and shows the distinctive “football” shape with a sclerotized ventral area encircling the genital field. Panel E and F represents *Albia*, a mite with a bluish hue and spherical in shape like *Mideopsis*. *Lebertia quinquemaculosa* is seen in panels G and H and is one of the larger mites collected, with an idiosoma of approximately 1 mm in diameter, and usually having five distinctive orange splotches that were readily identified on their dorsum; indeed, *L. quinquemaculosa* were often the target of collections on non-counts days because they were so readily identified and therefore became the subject of subsequent physiological experiments. Panels I and J represent *Neumania*, the second most abundant mite collected in Blue Heron Lagoon. Panels K and L represent *Mideopsis* that are the “sphere”-shaped water mites with the distinctive red and black coloration seen ventrally. *Axonopsis* (panels O and P) are among some of the smaller mites found in Blue Heron Lagoon. *Krendowskia* (panel Q and R) is a mite that closely resembles *Arrenurus* but has distinct coxal plates, observed in panel R. The coxal plates in *Krendowskia* do not form a straight line across the venter but have a V-shaped presentation. Panels S and T represent *Hydrachna*, a mite that has a reddish coloration. *Koenikea* is represented in panel U and V and can be mistaken for *Mideopsis* due to its spherical

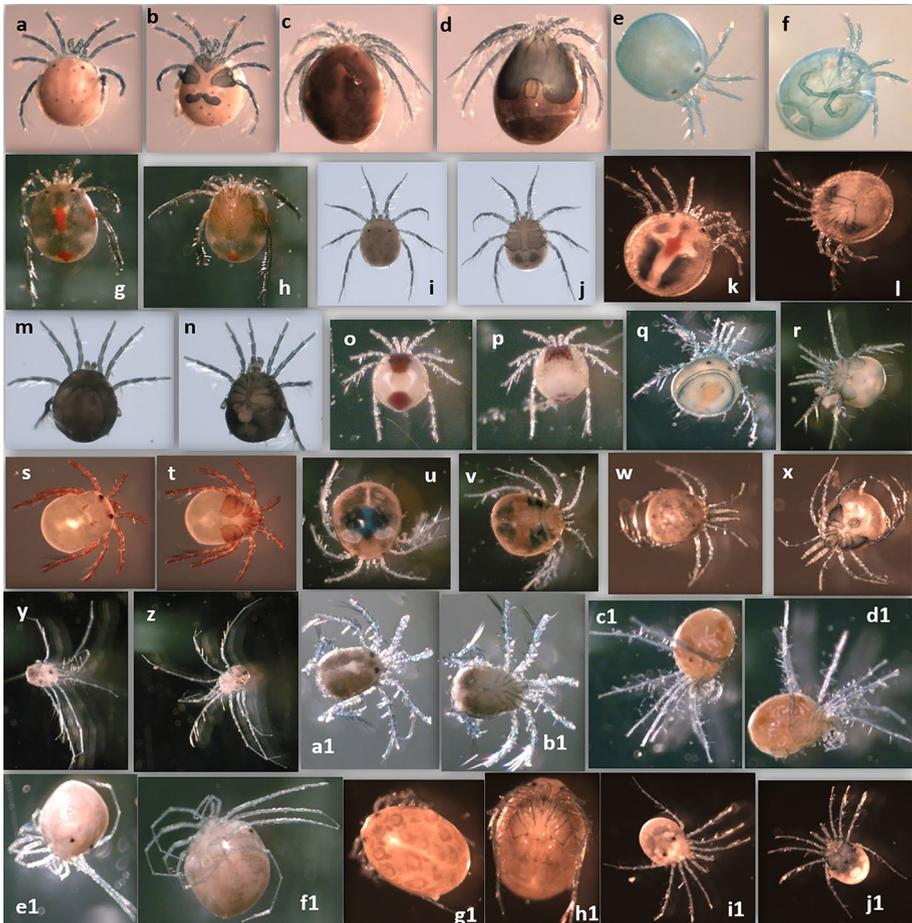


Fig. 2 The biodiversity of water mites in Blue Heron Lagoon, Belle Isle, Detroit. Mites were photographed to represent both dorsal and ventral views. Mite DNA was then extracted and molecular barcodes were obtained for representative mite genera. **a, b** *Arrenurus* deuteronymph, **c, d** *Oxus*, **e, f** *Albia*, **g, h** *Lebertia quinquemaculosa*, **i, j** *Neumania*, **k, l** *Mideopsis*, **m, n** *Arrenurus* adult, **o, p** *Axonopsis*, **q, r** *Krendowskia*, **s, t** *Hydrachna*, **u, v** *Koenikea*, **w, x** *Limnesia*, **y, z** *Unionicola*, **a1, b1** *Madawaska*, **c1, d1** *Hygrobatas*, **e1, f1** *Hydrochoreutes*, **g1, h1**, *Forelia*, **i1, j1** *Piona*

shape, but its coloration is quite distinct. *Limnesia*, seen in panels W and X has distinctive V-shaped coxal plates, as seen in Panel X. *Unionicola*, seen in panels Y and Z, is recognized by its characteristic “spider” like legs that are very long compared to its body size when extended. *Madawaska*, in panels A1 and B1, has a distinctive bend in its fourth leg. In panels C1 and D1, *Hygrobatas* has coxal plates whose posterior margins are in a straight line that aligns with the anterior edge of its genital field. Panels E1 and F1 illustrate a rarely seen mite in our collections, *Hydrochoreutes*. *Forelia* is seen in panels G1 and H1, and this mite was observed in winter collections. *Piona*, illustrated in panels I1 and J1, has unusually large palps in proportion to its body and represents a large group with potentially many species.

DNA barcodes of water mites from Blue Heron Lagoon

A curated neighbor-joining tree of sequences of COI DNA barcoded water mites from our collections in Blue Heron Lagoon is illustrated in Fig. 3. Genera such as *Arrenurus* and *Lebertia* can be observed as having several branches that differed from each other by more than 5%, suggesting the possibility of several species within those genera at Blue Heron Lagoon. We identified the upper branch of *Lebertia* as the large lake dwelling species *L. quinquemaculosa*. Publication of these *L. quinquemaculosa* sequences (submitted by us as GenBank accession IDs MG811661, MG811665, and MG811666) represent the first species level COI barcode in GenBank for *L. quinquemaculosa* since its description in 1929 by Ruth Marshall (Marshall 1929). *Lebertia* n. sp. (accession IDs MG811664, MG811684, MG811685) has a barcode differing by > 10% from any previously reported barcode and may likely represent a new species of *Lebertia* that has not previously been described (in preparation). Branches for *Albia* (MG773263), *Madawaska* (MG773265), *Axonopsis* (MG835761), and *Hydrochoreutes* (MG773264), marked in the tree by asterisks, represented, at the time of submission of these sequences to GenBank, the first publicly available COI barcodes for these genera.

BLAST analysis of representative water mite DNA molecular barcodes

The results of BLAST analysis of representative sequences of each branch of the neighbor-joining tree in Fig. 3 are listed in Table 1 and showed (at the time of submission of our sequences to GenBank) that only seven were represented in GenBank by an above 97% match. Among these seven matches, only one, *Arrenurus*, was identified in GenBank to genus level; two are matched with sequences identified by the correct family (Limnesiidae and Unionicolidae), and the other four only as Trombidiformes. The nearest genus level matches in the latter group are < 83% identical to our identified sequences. Other representative barcodes matched a sequence in GenBank mostly in the 79–89 percentile range, with the highest being 89% (*Mideopsis*) and the lowest 79% (*Hygrobatas*). Of the 20 representative sequences illustrated in Table 1, the nearest match in the GenBank database for seven of them was a genus or family other than the one we identified; however, in all such cases the pairwise identity was less than 85%, including *Neumania* mismatched with *Pionidae* (83%), family level identification *Aturidae* mismatched with *Hydraphantidae* sp. (85%) and *Hydrochoreutes* mismatched with *Piona variabilis* Koch (83%). For two GenBank family level matches in the 85–90% range, our genus identifications (*Piona* and *Mideopsis*) are consistent with the previous family identifications.

Relative abundance of water mite genera in Blue Heron Lagoon, Belle Isle Detroit

Figure 4 shows the frequency of water mite taxa among specimens sampled in timed effort collections, as described in the methods, at Blue Heron Lagoon, Belle Isle from July 2016 through June 2017. Analysis of 947 specimens comprising 15 genera (representing 11 families) and one family level (*Aturidae*) taxon revealed taxonomic and seasonal variation in

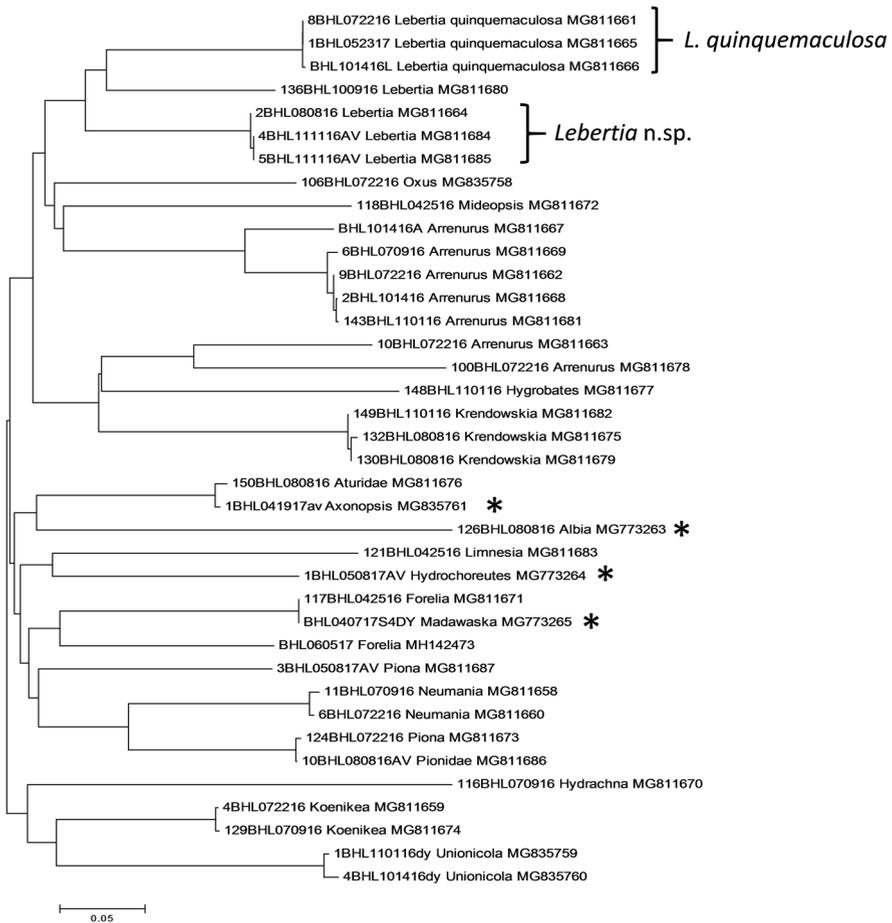


Fig. 3 Water mite genetic diversity of Blue Heron Lagoon, Detroit MI. Neighbor-joining tree showing sequence relationships among 38 water mite cytochrome oxidase I (COI) nucleotide sequences. The final dataset analyzed 625 positions and was constructed using MEGA6 (Tamura et al. 2013). Three branches of *Lebertia* sequences are present, of which two represent *Lebertia* species that will be compared in detail in another publication, naming the new species represented here only as *Lebertia* n. sp. Sequences indicated by asterisks (*) represent genera for which no genus level COI barcode had been published in GenBank prior to our submission of the sequences. The name of each branch includes the specimen archive number, the morphologically identified taxon, and the GenBank accession number of the submitted sequence

water mite abundance (Fig. 4). Although most genera were represented by just one putative species, some (*Arrenurus*, *Lebertia*) were represented by multiple putative species.

In field samples, the most frequently observed genera were *Arrenurus* (35%), *Neumania* (31%), *Lebertia* (12%), *Hygrobatas* (6%) and *Oxus* (3%). These were followed by *Limnesia*, *Mideopsis*, *Forelia* with 2% each. *Hydrachna*, *Axonopsis*, *Unionicola*, *Koenikea*, *Aturidae*, *Piona*, *Krendowskia* and *Albia* were all under 1%. Unidentified mites, which are not represented in Fig. 4, accounted for 3% of the total specimens.

Table 1 Closest matches in GenBank to COI sequences of Blue Heron Lagoon water mites

RamLab sample ID	RamLab taxon ID	Closest ID in GenBank	% Identity	% Query
8BHL072216	<i>Lebertia quinquemaculosa</i>	<i>Lebertia</i> sp. KP979189.1	86	99
136BHL100916	<i>Lebertia</i>	<i>Lebertia</i> sp. HQ938822.1	87	100
9BHL072216	<i>Arrenurus</i>	<i>Arrenurus</i> sp. KF880883.1	99*	100
126BHL080816	<i>Albia</i>	Trombidiformes sp. KR103686.1	99* [†]	91
11BHL070916	<i>Neumania</i>	Pionidae sp. KM830315.1	83	99
124BHL072216	<i>Piona</i>	Pionidae sp. KJ084272.1	86	99
118BHL042516	<i>Mideopsis</i>	Mideopsidae sp. KR099932.1	89	84
132BHL080816	<i>Krendowskia</i>	<i>Krendowskia</i> sp. KX139056.1	85	100
148BHL110116	<i>Hygrobates</i>	<i>Hydrphanties</i> sp. JX838402.1	79	99
150BHL080816	Aturidae	Hydryphantidae sp. KP979119.1	85	99
116BHL070916	<i>Hydrachna</i>	Xylophanes FJ026855.1	79	84
121BHL042516	<i>Limnesia</i>	Limnesiidae sp. KR103869.1	99*	89
1BHL110116dy	<i>Unionicola</i>	Unionicolidae sp. KM830666.1	99*	100
106BHL072216	<i>Oxus</i>	<i>Sperchon</i> sp. KC263080.1	83	100
BHL060517	<i>Forelia</i>	Trombidiformes sp. KM827333.1	99* [‡]	100
117BHL042516	<i>Forelia</i>	Trombidiformes sp. KM824376.1	99* [§]	100
1BHL041917av	<i>Axonopsis</i>	<i>Piona variabilis</i> JN034781.1	85	99
4BHL072216	<i>Koenikea</i>	Acari sp. KX139058.1	89	100
BHL040717S4DY	<i>Madawaska</i>	Trombidiformes sp. KM824376.1	99* [¶]	100
1BHL050817AV	<i>Hydrochoreutes</i>	<i>Piona variabilis</i> JN034781.1	83	100

The COI sequence of the RamLab identified specimen was queried against GenBank using BLAST (Altschul et al. 1990). The % identity and query coverage of the top of the match list are shown. Specimens with % identity matches of >97% are highlighted with an asterisk *. Where the highlighted matched taxon identified only as Trombidiformes, a footnote provides the % identity for the best genus level match. Nearest genus level matches for specimens whose best GenBank match was identified only as Trombidiformes: [†]*Sperchon* sp. KC263080.1, 78%, [‡]*Hydryphanties* sp. JX838402.1 82%, [§]*Piona variabilis* JN034781.1, 83%, [¶]*Piona variabilis* JN034781.1, 83%

Water mite populations varied monthly as seen in Fig. 4, with *Arrenurus* being very common during the months of July and August (n=266), and *Neumania* being the second most common genus during those months (n=108). During the months of October and November the populations of *Arrenurus* plummeted (n=47) while *Lebertia* populations increased (n=73). *Neumania* populations remained similar (n=79). In February, most mite populations were in decline as only 52 mites were collected in total, with the most common being *Lebertia* (n=41) and *Forelia* (n=8). During April, *Neumania* increased in frequency (n=89). June showed an increase in *Arrenurus* (n=30) and *Neumania* (n=89).

Seasonal presence of additional animals collected and not included in the above timed effort collections species include specimens of *Hydrochoreutes* collected in May 2017, *Madawaska* collected in April 2017, *Unionicola* collected in October 2016, and *Hygrobates* in early November 2016.

Diversity analysis

The rank abundance curves in Fig. 5a illustrate seasonal changes in the richness of genera and the evenness of their respective relative abundance. The highest richness

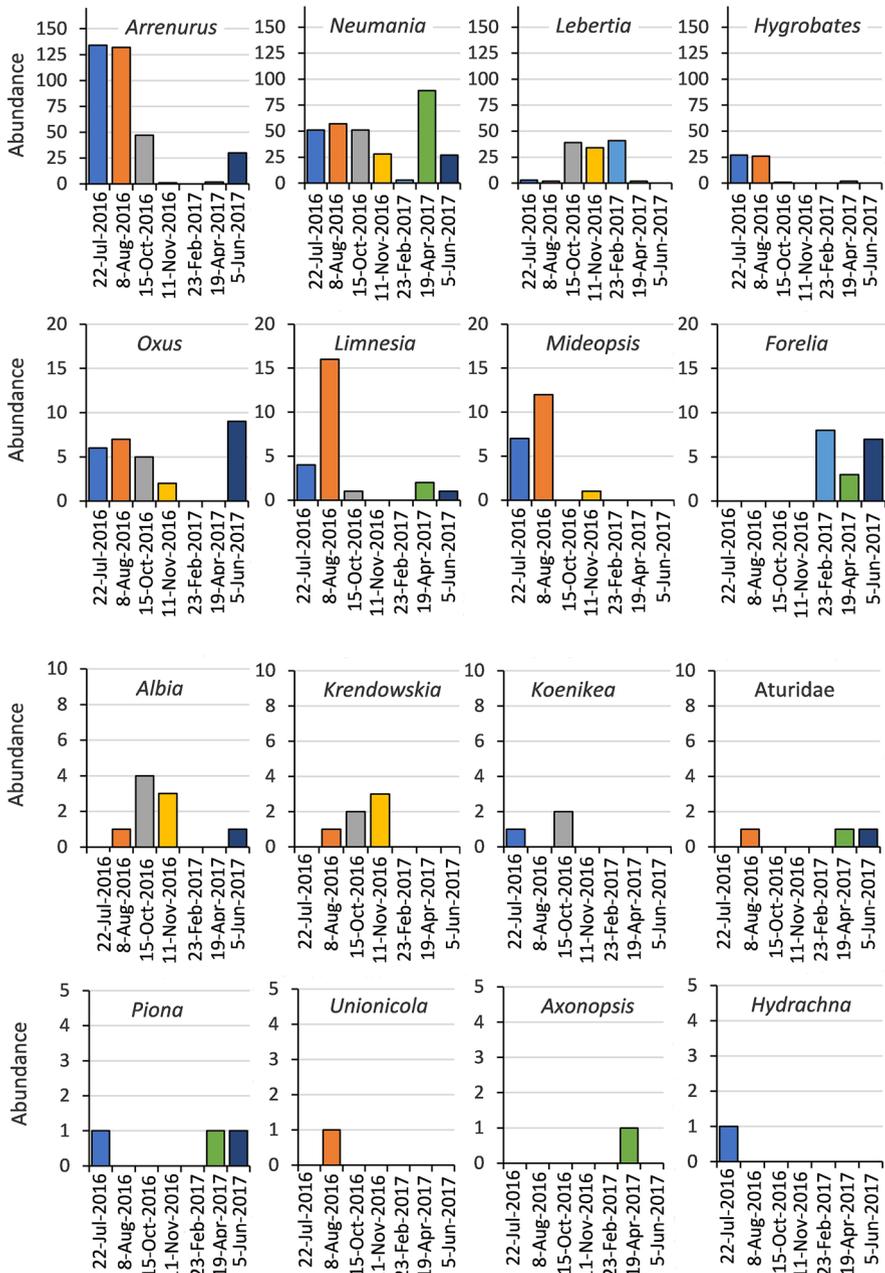


Fig. 4 Frequency of various water mite taxa collected in Blue Heron Lagoon. Water mites were sampled with comparable intensity on six collection dates from July 2016 to May 2017, counted, and identified where possible by morphological keys to genus. Graphs are arranged (left to right, and then by row) in the order of the overall frequency of each taxon. Each bar graph shows the frequency on the six collection dates with bars color-coded to assist in comparing graphs on various dates, as follows: 22 July 2016, blue; 8 August 2016, orange; 15 October 2016, gray; 11 November 2016, yellow; 23 February 2017, light blue; 19 April 2017, green; and 5 June 2017, dark blue. (Color figure online)

of genera was observed during April, July, August, and October (9 genera each), while the lowest richness was observed in February (3 genera). Cluster analysis of mite genera proportional abundances (Fig. 5b) indicated three temporal groups, clustering two summer months (July and August), two winter/early spring months (February and April; for convenience and in consideration of Michigan’s long winters, labeled “winter” in the illustrations), and a group that we are calling transition months June, a late spring month, clustered with November, and October.

Applying diversity analysis to these three clusters, the rarefied Shannon diversity index based on 155 individuals per cluster was highest during the transition months ($H=1.47$), lowest during winter months ($H=1.12$), and intermediate during the summer months ($H=1.35$) (Fig. 6).

The number of mite genera was greatest during transition months (13 genera), lowest during winter/early spring months (9 genera), and intermediate during summer months (11 genera). The number of mite genera predicted by multivariate estimators was greatest during transition months (15–19 genera), lowest during winter months (9–12 genera), and intermediate during summer months (11–17 genera) (Fig. 7). The number of shared genera observed was greatest between transition and winter months (7 genera) as well as between transition and summer months (7 genera), but lowest between summer and winter months (6 genera). The Sorensen and Morisita–Horn similarity indices show that the proportion of shared genera was greatest between transition and winter months (approximately 70–85%), lowest between summer and winter months (33–60%), and midway in shared genera between transition and summer months (60–70%) (Fig. 8).

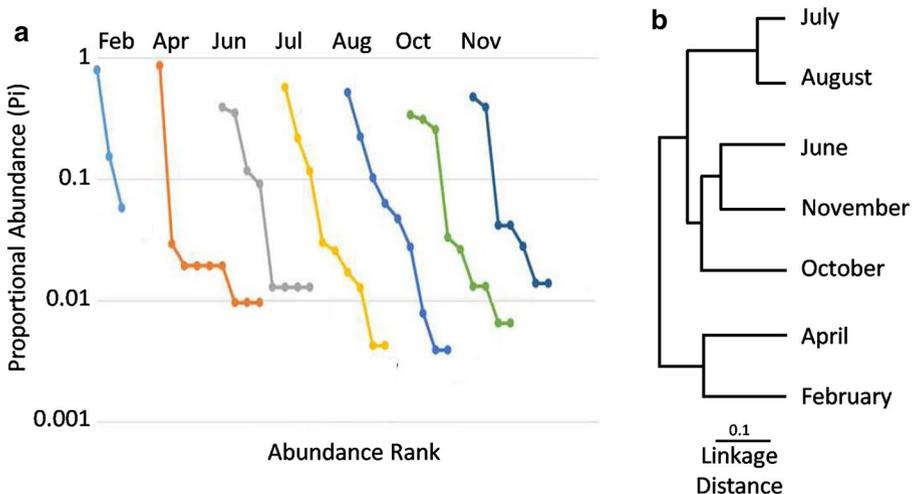


Fig. 5 Temporal proportional abundance and cluster analysis on water mite assemblages. **a** Rank-abundance curves showing proportional shifts in the composition of mite genera throughout the year. **b** Dendrogram showing three hierarchical clusters in the composition of mite genera throughout the year

Fig. 6 Rarefied Shannon diversity indices computed at 155 individuals for mite genera sampled during summer, transition and winter months. Bars denote a standard deviation

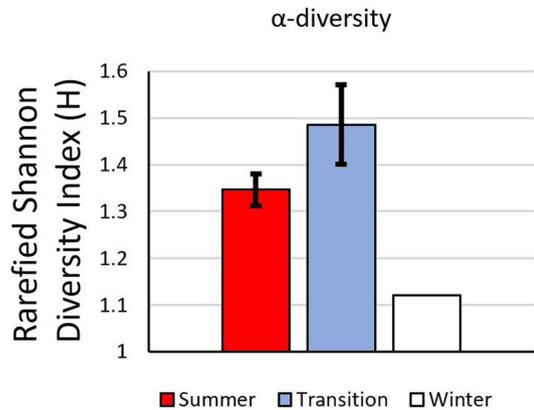
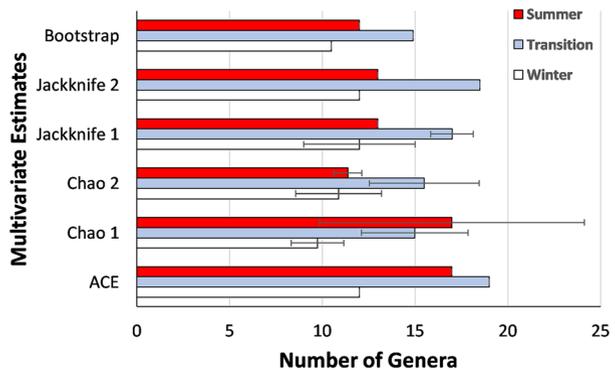


Fig. 7 The predicted number of mite genera during summer, transition, and winter months by means of multivariate richness estimators. Bars denote a standard deviation

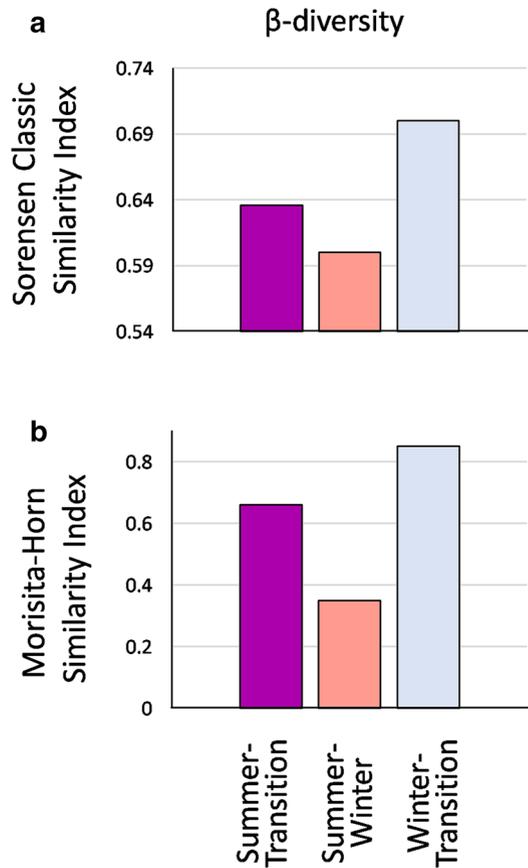


Discussion

This paper makes three significant contributions: (1) The source and methods for collection, identification, and determination of new genetic barcodes of water mite taxa that were not previously represented in GenBank are described, including two species-level barcode identifications for *Lebertia*, several genera not previously represented in GenBank at all, and novel barcodes (i.e., genetically distant from previously published barcodes) for several other water mite genera; (2) the first description of seasonal changes and richness of water mite community dynamics in the Detroit River is presented; and (3) in this discussion, analysis of seasonal changes in water mite diversity is proposed as supporting evidence for application of the intermediate disturbance hypothesis (IDH) to explain diversity in the temporal domain.

Whereas observations of molecular barcodes to species level are common for vertebrate species in the Great Lakes, the lack of such corresponding data for many invertebrate groups has been highlighted by Trebitz et al. (2015) and reinforced by many studies, such as Failla et al. (2016) that increased the representation of species level barcodes for chironomid larvae in western Lake Erie from 16% of larval operational taxonomic units (OTUs) to 42% and Vasquez et al. (2017) that added to the public databases the first water mite DNA barcodes for *Krendowskia* and *Koenikea*. Knowledge of what species of water

Fig. 8 Beta-diversity of mite genera between pairings of summer, transition, and winter months using the similarity indices **a** Sorensen Classic and **b** Morisita–Horn



mites are present is important for determining whether new species have recently invaded, understanding the food web role of these carnivores and parasites of aquatic insects and other organisms, and determining their utility as bio-indicators. Over the course of this study (July 2016 to June 2017), *Arrenurus*, *Neumania*, and *Lebertia* were by far the most frequently collected mites found in Blue Heron Lagoon; however, water mites of many genera could be collected from this environment during all possible collecting months, ice-cover on Blue Heron Lagoon notwithstanding, with *Lebertia* especially common throughout the late fall and winter. A total of at least 17 genera of water mites are present in Blue Heron Lagoon, including four genera for which the genus level COI barcodes are the first to have appeared in GenBank at the time the barcodes were submitted. While additional water mite COI barcodes are also present in the Barcode of Life database, most of those are identified only to family level or higher or are private, not publicly accessible sequences, which are therefore of less use for the applications of barcodes that we have highlighted in this paragraph.

The diversity of water mite genera in Blue Heron Lagoon indicates a healthy environment in the lagoon. Analysis of aquatic invertebrates, including mayfly populations, chironomid abundance and deformities, and oligochaete populations, have been used in past assessments of ecosystem health of the Detroit River AOC (of which Blue Heron Lagoon

is a part), showing great improvement of the river since the 1960s (Hartig et al. 2009). However, no environmental assessment of the Detroit River has used analysis of water mite populations in spite of suggestions by a number of authors (Goldschmidt et al. 2016; Klemm et al. 1990; Miccoli et al. 2013) that a range of water mite species may be useful in that regard. *L. quinquemaculosa*, which we frequently observed in Blue Heron Lagoon, is known to be intolerant of organic wastes (Klemm et al. 1990). On a 0–5 scale on which 5 is the category of the most tolerant organisms, *L. quinquemaculosa* was characterized as “intolerant” (category 1) (Klemm et al. 1990). In comparison, multiple species of *Arrenurus* were “facultative tolerant” (category 2 or 3), *Neumania* were “intolerant” (category 1), and several *Hygrobatas* species were highly tolerant of organic wastes (category 4). The frequent observation of *Lebertia* specimens, including *L. quinquemaculosa*, in the winter months, and *Neumania* during the rest of the year, in Blue Heron Lagoon indicates that on this organic waste tolerance scale this lagoon has excellent environmental conditions for freshwater life throughout the year.

Diverse transition periods revealed in community structure analysis

Analysis of the temporal proportional abundance of mite genera and cluster analysis of those data suggests the occurrence of three temporal assemblages (Fig. 5): summertime and wintertime communities are connected by diverse transitional periods (Figs. 6 and 8). The unexpected observation, supported by several different types of community analysis (Shannon diversity, Fig. 6, and beta diversity, Fig. 8), is that the months of June, October, and November represent especially diverse transition months. The multivariate estimators of population diversity (Fig. 7) indicate that the lower observed winter and summer richness is within the predicted range, while the transition months are, if anything, even more diverse than we collected since the number of genera during the transition periods is predicted by all estimators to be greater than the 13 genera we observed during those months. These identified transition months in water mite community structure may reflect the changes in temperature and precipitation as relatively stable cold winter weather changes to summer and can be compared to the IDH (Connell 1978; Wilkinson 1999), but applied to the temporal domain. Low wintertime alpha diversity may result from extreme environmental conditions (e.g. freezing temperatures, aquatic vegetation die-off, low resource availability, etc.) while the moderate summertime alpha diversity likely reflects the dominance by a few highly competitive mite genera that effectively exclude other members of the community (Gause 1934; Grinnell 1904). Mite community diversity may be maximized during transitional months when weather extremes are neither rare nor frequent.

Application of the IDH in the temporal domain has been modeled and helps explain how periodic disturbances can help maintain stable diverse populations in the presence of changing conditions, as described in Roxburgh et al. (2004). The present study is the first time the IDH has been used to describe the seasonal changes in the organismal diversity of mite assemblages. The high diversity during transitional months may be an integral feature of mite community dynamics in temperate ecosystems. Comparably, the latitudinal diversity gradient of Oribatid mites increases away from the tropics (Caruso et al. 2019). With the identification of these temporal changes in mite assemblages, future field studies can evaluate the local environmental disturbances that contribute to these transitions.

Blue Heron Lagoon appears to be a good location for further studies of *Arrenurus* and *Lebertia*. *Arrenurus* is the most species-rich genus found in North America with over 100 species described so far, and with the possibility of hundreds more (Cook 1976). *Arrenurus* specimens exhibited multiple barcode branches differing in sequence by >9% and therefore likely to represent different species. This may indicate a great potential for comparative studies of readily distinguished *Arrenurus* species within this environment. *Lebertia* are especially interesting due to their relatively large size, and they have been the subject of a variety of studies beyond purely taxonomic or molecular descriptions, including diet (Vasquez 2017), circadian rhythms (Schmidt and Muller 1967), role as an ectoparasite on chironomids (Martin 2004; Efford 1963), and reproduction (Bankowska et al. 2016). Descriptions of *Lebertia* species by Gerecke (2009) and collaborators (Esen et al. 2013) indicate 136 species of *Lebertia* worldwide. In North America, various *Lebertia* subgenera and species have been described by Cook (1974), Smith et al. (2010), Marshall (1927), and Crowell (1960). Our identification and barcoding of *L. quinquemaculosa* in Blue Heron Lagoon is a new record for this species in waters contiguous with the Great Lakes. Other observations of *Lebertia* in North America are reviewed in Vasquez et al (2017).

Earlier research around the Great Lakes played a critically important role in understanding the systematics of water mites (Cook 1954, 1967, 1974, 1976) but the application of molecular techniques, as in this study, may enable a greater appreciation of the role of water mites in Great Lakes ecology. Given their diversity, usefulness as bio-indicators and ecological roles as predators and parasites of chironomids, mosquitoes, and other microinvertebrates, the present work provides an important platform on which future work using water mites in North America can be carried out.

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Author contributions AAV conceived the study, collected the specimens, conducted taxonomic and molecular work and wrote the manuscript. MSQ and XNW assisted in the collection of specimens and molecular work. VC-G assisted with diversity indices analysis and statistics including writing and editing the manuscript. JLR contributed to the conceptualization of the work and to the writing and editing of the manuscript.

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