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Water Mite Diversity (Acariformes: Prostigmata: Parasitengonina: Hydrachnidiae) from Karst Ecosystems in Southern of Mexico: A Barcoding Approach

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Abstract: Water mites represent the most diverse and abundant group of Arachnida in freshwater ecosystems, with about 6000 species described; however, it is estimated that this number represents only 30% of the total expected species. Despite having strong biotic interactions with their community and having the potential to be exceptional bioindicators, they are frequently excluded from studies of water quality or ecology, due to actual and perceived difficulties of taxonomic identification in this group. The objective of this study is to use the variations in the sequences of the mitochondrial cytochrome oxidase subunit I (COI), also known as the DNA barcodes region, as a tool to assess the diversity of water mites at 24 sites in the Yucatan Peninsula of Mexico. We found 77 genetic groups or putative species corresponding to 18 genera: *Arrenurus*, *Atractides*, *Centrolimnesia*, *Eylais*, *Geayia*, *Hydrodroma*, *Hydryphantes*, *Hygrobates*, *Koenikea*, *Krendowskia*, *Limnesia*, *Limnochares*, *Mamersellides*, *Mideopsis*, *Neumania*, *Piona*, *Torrenticola*, and *Unionicola*. This was significant, since there are only 35 species described for this region. Furthermore, this molecular information has allowed us to infer that there are characteristic assemblies per site. These data will facilitate the incorporation of water mites in different studies while the curatorial work continues to assign a Linnaean name.

Keywords: COI; Yucatan Peninsula; assemblages; richness; Acari

1. Introduction

Water mites belong to the Hydrachnidiae subcohort and represent the most important, abundant, and diverse group of the Arachnida in freshwater ecosystems [1,2]. There are about 6000 named species, with 1300 of them reported from the Neotropics. According to Goldschmidt [3], the neotropical water mite fauna is far from being completely described, and approximately 5440 species could reasonably be expected in this area.

Mexico is a mega-diversity country due to its position in a transition region between the Nearctic and Neotropical zones and its complex physiography [4]. As a result, it is the country in the world with the second highest number of ecosystems and the fourth in terms of biodiversity [4]. In relation to aquatic environments, we know only a small fraction of their biological diversity. Regarding water mites, 317 species have been described and some reported here in the last 40 years [5]. Only 35 were from the Yucatán Peninsula that comprises three Mexican states (Quintana Roo, Yucatan, and Campeche) [6–8].

The Yucatan region includes one of the world's largest karstic aquifers and that represents a mosaic of different geochemistry and hydrogeologic properties on its water ecosystems [9,10]. For example, the Cenote Azul, located in the southern part of the Yucatan Peninsula (18.647 N and 88.412 W, Datum WGS84), is a unique extreme environment, characterized by a high sulfate and strontium content

water [10]. Lake Bacalar, also located in the south, hosts the largest living freshwater microbialites in the world [11,12] and has a rich mite fauna, which is still unknown [13].

According to Cook (1980), we were far from knowing all the local water mite diversity in the neotropics, and this situation has not improved significantly over the last 40 years. Other authors have observed that neotropical water mite fauna shows regional diversification, and a high degree of richness and endemism should be expected in this region [1,3].

The taxonomy of water mites is difficult, and systematics is constantly subject to changes [14–16], first, due to the complex life cycle composed of three active stages: parasitic larva, depredatory deutonymph, and adult and three resting stages, namely prelarva, protonymph, and tritonymph, plus the egg [2]. Some groups, such as adults arrenurids, also present a strong sexual dimorphism, where males and females are completely different morphologically. In other cases, this dimorphism is visible in the modification of the legs IV for males. Finally, the diagnostic characteristics, such as setaes, coxal groups, acetabular plate, glandularias, or palps are difficult to identify without taxonomic training. Due to these challenges, many synonyms, cryptic species, subspecies, and "forms" with questionable identity exist in the literature [6,14,17].

The application of molecular biology techniques adds new characters to taxonomy. A particular region of the mitochondrial COI (cytochrome c oxidase I) gene, one of the groups known as DNA barcode region, is the most common sequence used in water mite taxonomy research. Public databases, including the Barcode of Life Database (www.boldsystems.org) or GenBank (www.ncbi.nlm.nih.gov), and the use of new bioinformatic tools represent a breakthrough in species identification [18,19]. On the other hand, these molecular data allow us to understand, from another perspective, not only the identity of species, but also ecological relationships that exist between these animals. It is also another important character for new species descriptions [20,21] that can solve problems related to cryptic species complexes [14,15], and matching of different development stages from eggs to adult males and females despite their morphological differences [16,21–24].

Additionally, DNA barcoding and the BOLD database (boldsystems.org) can be used to obtain a preliminary approximation of distribution patterns, species assemblages, richness, and diversity among other analysis [25]. The Barcode Index Number (BIN) is a fast-computational algorithm based on differences of the COI fragment. It is a unique Operational Taxonomic Unit (OTU) that highlights a putative species, assigning an exclusive code composed of alphanumeric characters [26]. The BIN system provides information about specimens with their associated metadata (taxonomy, distribution, images, sequences, collector, identifier, and institution where the voucher/specimens is deposited) [26]. This system has been used with success in diverse invertebrate surveys, biodiversity assessments, and species delimitation [13,25,27,28]. Currently there are 77,666 Trombidiformes records in the database where Hydrachnidia is a subcohort.

The aim of this study was to assess water mite diversity in different water bodies from the Center to the Southern Yucatan Peninsula, using DNA barcoding and the subsequent BIN representing each OTU, and their correspondence with identified morphotaxa, as the main approach.

2. Materials and Methods

2.1. Collection of Samples

Data were mined from both BOLD corresponding to previous published studies by the authors [13,23] and unpublished data from a last sampling survey carried out in April and August corresponding to the dry and rainy seasons. By the end, all data represented 24 sites (Table 1) from Yucatan Peninsula (PY), Mexico (Figures 1 and 2). All the samples were collected according to the methods in earlier studies [23], with the exception of two systems: Acapulquito and Palmar, where the collection was carried out by using manual nets with a mesh of 100 μm.

Table 1. Collection locations and Barcode Index Numbers (BINs) associated.

Number	Site	Lat N	Long W	BINs
1	Acapulquito	18.4321	88.5312	11
2	El palmar	18.4407	−88.5273	6
3	Cenote Azul	18.651	−88.4098	14
4	Cenote Cocalitos	18.652	−88.408	21
5	Cenote Escuela Normal	18.651	−88.409	9
6	North Bacalar Lake	18.9176	−88.171	14
7	Cenote Pucte 1	19.079	−87.994	11
8	Cenote Pucte 2	19.091	−87.994	9
9	Cenote el Toro	19.098	−88.021	2
10	Ramonal	19.3921	−88.6242	10
11	Cenote Sijil Noh Ha	19.475	−88.052	3
12	Cenote Chancah Veracruz	19.486	−87.988	4
13	Cenote del Padre	19.604	−88.003	6
14	Minicenote	19.607	−87.989	2
15	Cenote Tres Reyes 1	19.668	−87.881	3
16	Cenote Tres Reyes 2	19.692	−87.877	6
17	Santa Teresa	19.723	−87.813	2
18	Chichancanab	19.924	−88.7708	7
19	Cueva de las serpientes	19.93	−88.806	1
20	Cenote km 48	19.943	−87.794	6
21	Chunyaxche Lagoon 1	20.042	−87.581	3
22	Chunyaxche Lagoon 2	20.06	−87.576	12
23	Muyil Lagoon 1	20.069	−87.594	8
24	Muyil Lagoon 2	20.075	−87.607	4

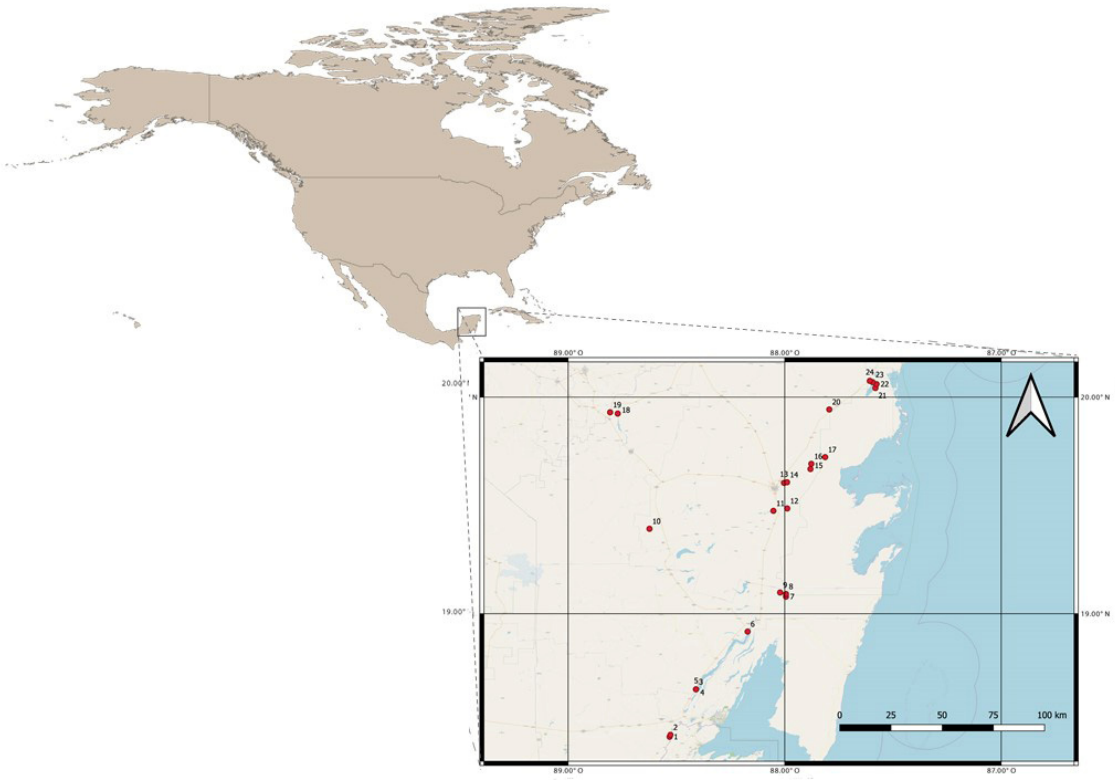

Figure 1. Location of the studied karstic systems. Names and coordinates for each site are in Table 1.

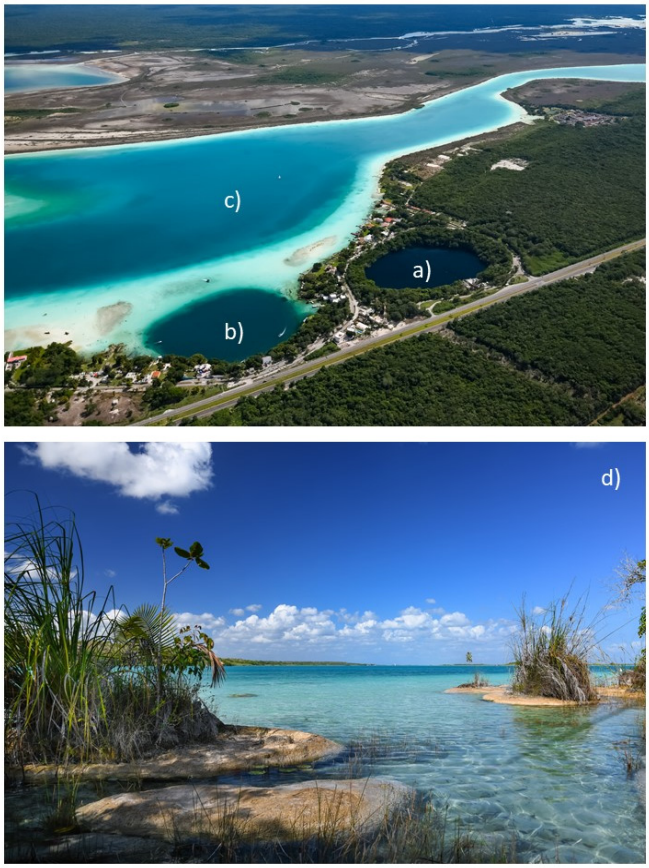

Figure 2. Examples of some sampled localities: (**a**) Cenote Azul, (**b**) Cenote Cocalitos, (**c**) Bacalar lake, and (**d**) large microbialites from Bacalar lake. Photos taken by ©HBahena/ECOSUR.

2.2. Specimen Preparational Analysis

In the laboratory, the fixed samples were viewed under a stereo microscope, and water mites were removed from each one. Representatives of each morphologically distinct group were separated and stored in 5 mL vials with 4 mL of 96° ethanol. All the water mites were identified to genus, using published keys [2,14,28]. All mites were photographed in a stereo microscope Zeizz Stereo Discovery with an Eos Rebel T3i camera.

2.3. DNA Extraction and Amplification

Whenever it was possible, five individuals of each genus were selected for genetic analysis. The whole water mite specimens were placed into 96-well plates, and DNA extraction was carried out by using a standard glass fiber method [29]. After the DNA extraction, the vouchers were recovered and preserved in Koenike's solution for future curatorial labor and deposited in the Reference Collection at El Colegio de la Frontera Sur, Unidad Chetumal (ECOCH-Z-10339-10364).

The PCR mixtures contained a final volume of 14 µL and were prepared as follows: 2 µL of Hyclone ultra-pure water, 6.25 µL of 10% trehalose (previously prepared: 5 g D-(+)-trehalose dehydrate, in 50 mL of total volume of molecular grade ddH2O), 1.25 µL of 10X PCR buffer, 0.625 µL of MgCl2 (50 mM), 0.0625 µL of dNTP (10 mM), 0.125 µL of each primer (10 µM), 0.06 µL of Platinum Taq DNA polymerase, and 3 µL of DNA template. All specimens were amplified with the zooplankton primers (ZplankF1_tl and ZplankR1_tl). The reactions were cycled at 94 °C for 1 min, followed by five cycles of 94 °C for 40 s, 45 °C for 40 s, and 72 °C for 1 min, followed by 35 cycles of 94 °C for 40 s, 51 °C for 40 s, and 72 °C for 1 min, with a final extension of 72 °C for 5 min. PCR products were visualized on 2% agarose gel (E-Gel 96 Invitrogen); finally, positive PCR products were selected for sequencing.

PCR products were sequenced, using a modified BigDye © Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA, USA), and sequenced bidirectionally on an ABI 3730 capillary sequencer at Eurofins Scientific. Sequences were edited by using Codon Code v.3.0.1 (CodonCode Corporation, Dedham, MA, USA). Sequence data, trace files, collection data, and primer details for all specimens are available within the public dataset DS-YUCWM through the public data portal of the Barcode of Life Data Systems (www.boldsystems.org) and in GenBank (www.ncbi.nlm.nih.gov).

2.4. Sequencing and Data Analysis

All sequences that met minimal quality standards (≥500 bp, without ambiguous bases or stop codons) were assigned to a BIN [19,26]. These BINs are considered putative species or OTUs [13].

The analysis of all sequences with a BIN assignment was conducted by using MEGA v.6. We constructed Neighbor Joining trees for the most families with large numbers of BINs (Arrenuridae, Limnesiidae, Unionicolidae, and Hygrobatidae). The simplified trees were prepared by using Figtree v1 4.4.

A Jaccard index and a dendrogram were calculated with Excel software, to assess beta diversity and the similarity of water mites' BINs among the 24 locations.

3. Results

A total of 607 water mite sequences representing 77 BINs were obtained. These corresponded to 13 families: Anisitsiellidae, Arrenuridae, Eylaidae, Hydrodromidae, Hydryphantidae, Hygrobatidae, Krendowskiidae, Limnesiidae, Limnocharidae, Mideopsidae, Pionidae, Torrenticolidae, and Unionicolidae.

The number of BINs per site varied from one at Cueva de las serpientes to 21 at Cenote Cocalitos (Table 1).

We observed a correspondence between the BINs and the morphospecies for all the mite specimens. In Figure 3, we can see the correspondence between BINs and representatives of the Krendowskiidae family and *Limnesia* genera. In most cases, we matched molecularly and morphologically each BIN to a genus level, except for the following 15 that could only be assigned to families: Torrenticolidae, Limnesiidae, Hygrobatidae, Pionidae, Unionicolidae, and Eylaidae; and three BINs pertaining to Trombidiformes (Table 2).

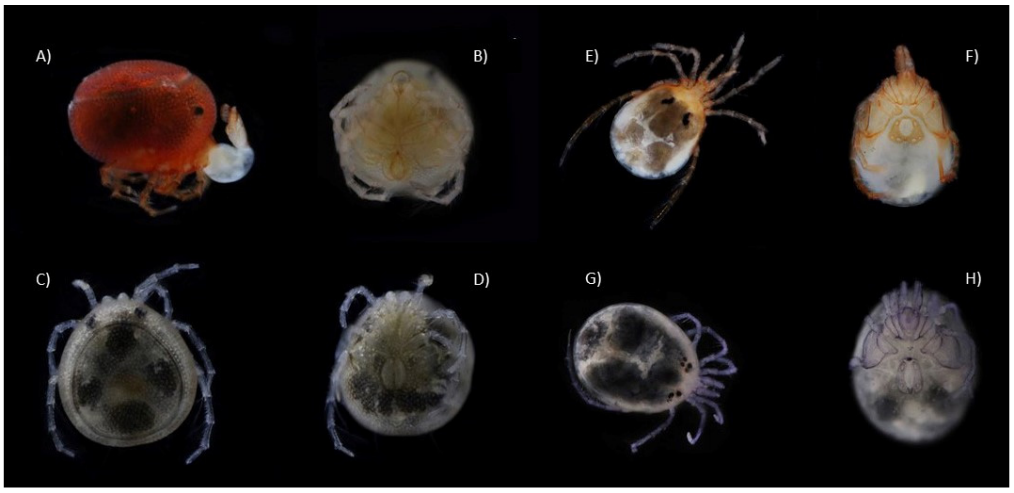

Figure 3. Members of Krendowskiidae family and *Limnesia* genus. (**A**,**B**) Lateral and ventral view of *Geayia* BIN ACT6195; (**C**,**D**) Dorsal and ventral view of *Krendowskia* BIN ACX8435; (**E**,**F**) dorsal and ventral view of *Limnesia* BIN ACY7380; and (**G**,**H**) dorsal and ventral view of *Limnesia* BIN AEA5595.

Table 2. Summary of taxa identified, BIN, and location.

Family	Genera	BIN	Location
Limnocharidae	*Limnochares*	ADI4862 *	3
		AEA4515 *	14
		AEB4511 *	24
		ACY6840	3, 6, 4
Hydrodromidae	*Hydrodroma*	ADF3732 *	3, 4, 23, 6, 18, 2, 20, 8.
Hydryphantidae	*Hydryphantes*	AEA5005 *	3
Torrenticolidae	*Torrenticola*	AEA7372 *	1
	Unknown genera	AEA4395 *	1
Limnesiidae	*Limnesia*	AEA5595 *	13, 6, 18, 7, 12.
		AEA6471 *	10
		ACX7759	5,4
		ACY7380	19, 5, 22, 4, 2, 6
	Centrolimnesia	AEA3914 *	9, 8, 16, 17
	Unknown genera	AEA4382 *	16, 9
Krendowskiidae	*Krendowskia*	ACX8435	20, 13, 5, 16, 24, 18, 6, 4
	Geayia	ACT6195	1
Mideopsidae	*Mideopsis*	AEA6512 *	1
		ACX8679	20, 13, 18, 5, 4, 24, 11, 23, 22, 8.
		ACY7169	7, 4, 22, 5.
	Unknown genera	AEB4633 *	12
Hygrobatidae	*Hygrobates*	AEA3689 *	1
		AEA3690 *	2
		AEA3924 *	1, 2
		ACX7887	3, 18
		ADO7098	6
	Atractides	ACX7786	5, 4
	Unknown genera	AEA4089 *	23
		AEA5236 *	21, 22, 23
Pionidae	*Piona*	AEB1670 *	6
		ACX8296	13, 12, 3, 6, 4, 23, 24, 7.
	Unknown genera	AEA4809 *	22
Unionicolidae	*Unionicola*	ACX8035 *	4
		AEB4634 *	8
		ACX8034	5, 4, 3, 7, 8, 14
		ACX9008	4, 5, 6
		ADM7936	21, 22, 23, 3
		ADP1665	4, 7, 22, 6
	Koenikea	ADI2928 *	3
		ACY7384	4, 5, 22
		ADI3114	2, 22, 6, 20, 3, 18, 8, 1.
	Neumania	AEA8101 *	20, 7, 10
		AEA5358 *	10
		ACY6829	6, 4
	Unknown genera	AEA4829 *	22, 8, 16
		AEA6062 *	23, 22.
		AEA6668 *	16
		AEA7951 *	16
		AEB1594 *	8
		ACY7381	4
		AEA3726 *	7
		AEA4514 *	1

Table 2. *Cont.*

Family	Genera	BIN	Location
Eylaidae	*Eylais*	ADD9174 *	4
	Unknown genera	AEA4696 *	15
		AEA5669 *	15
Arrenuridae	*Arrenurus*	ACX8462 *	4
		ACX8780 *	4, 2, 1
		ADI3752 *	3
		AEA3972 *	10
		AEA7182 *	7, 20
		AEA7842	10
		AEA7843 *	10
		AEA7844 *	1
		AEA8234 *	10
		ACL2418	4
		ACX8463	6, 4, 18, 23, 3, 12, 11, 13.
		ACX8464	4, 3, 13, 10, 6, 18.
		ACX8788	5
		ACY6809	7, 4, 21, 22, 24, 4,3
		AEB7095	1
		ADI4458 *	3
		AEA4828 *	17
Anisitsiellidae	*Mamersellides*	AEA6955 *	10
		AEA6956 *	10
Unknown		AEA4343 *	11
		AEA3823 *	15
		AEB1898	8

Localities are the same as Table 1. * Unique BINs in the Barcode of Life Database (BOLD) system.

3.1. Water Mite BINs Richness

Unionicolidae was the most diverse and abundant family, with 20 BINs and 230 sequences distributed among three genera, which were identified as *Unionicola, Koenikea,* and *Neumania*, and unidentified specimens. Fifty percent of the BINs of this family appear to have a restricted distribution inhabiting only one locality, while the other half was found in two to eight localities as *Koenikea* with the BIN ADI3114 (Figure 4 and Table 2).

The Arrenuridae was the second most diverse family, with 123 sequences and 17 BINs. All of them belonged to the genus *Arrenurus*. For nine BINs from this group, it was possible to correlate males and females and nymphs for three of them (Figure 5. Most of the BINs apparently inhabit only one location, and only three of them seem to have a wide distribution: ACX8463, ACX8464, and ACY6809 (Figure 6 and Table 2).

The Hygrobatidae and Limnesiidae families each had a moderate number of BINs. Hygrobatidae was represented by 48 sequences corresponding to eight BINs; two of them could be identified to genera *Hygrobates* and *Atractides*, and two more BINs could be identified only to family. Most of the Hygrobatidae occur only in one or two localities (Figure 7 and Table 2).

The Limnesiidae are represented by 68 sequences and six BINs, with four of them identified as *Limnesia*, one *Centrolimnesia*, and one unidentified genus. More than 80% of the limnesiids occurred in in two or more localities (Figure 8 and Table 2).

Other, less diverse families were the Limnocharidae, represented by nine sequences and four BINs, all of them *Limnochares*. Each BIN was found in a single locality, except for ACY6840, which was found in three close systems: Cenote Azul, Cenote Cocalitos, and North Bacalar Lake. Mideopsidae was represented by 36 sequences clustering in four BINs, with three of them from *Mideopsis* and the

other one identified only at the family level; *Mideopsis* BIN ACX8679 seems to have a wide distribution, as it was found in ten localities (Table 2).

Pionidae and Eylaidae were composed of three BINs and were each represented by one genus, *Piona* and *Eylais*, respectively; however, in both families, there were BINs with no genus assignment. In the case of Eylaidae, each BIN inhabited one system, while *Piona* ACX8296 was found in eight localities (Table 2).

Krendowskiidae was represented by two genera, *Geayia* and *Krendowskia*, with 32 sequences and two BINs (Figure 3). Krendowskia ACX8435 was widely distributed. Hydrodromidae was represented by one BIN and 22 sequences belonging to *Hydrodroma* genus. This OTU is widely distributed in eight systems in the sampled area, and all the morphotypes corresponded with one putative species.

Hydryphantidae was a singleton of the genus *Hydryphantes*. Finally, there were five sequences represented by three BINs that belonged to the order Trombidiformes. These individuals were nymphs, which are not included in any taxonomic keys. They cannot be further identified until an adult can be sequenced, as for *Arrenurus* specimens (Figure 5).

From the 77 BINs, 51 were sequenced for the first time and appear as unique in the BOLD database (Table 2). Only four BINs had a wide distribution, from Neotropical Mexico to Eastern– Central Canada. These are the *Unionicola* ADP1665, *Arrenurus* ACL2418, *Geayia* ACT6195, and *Piona* ACX8296.

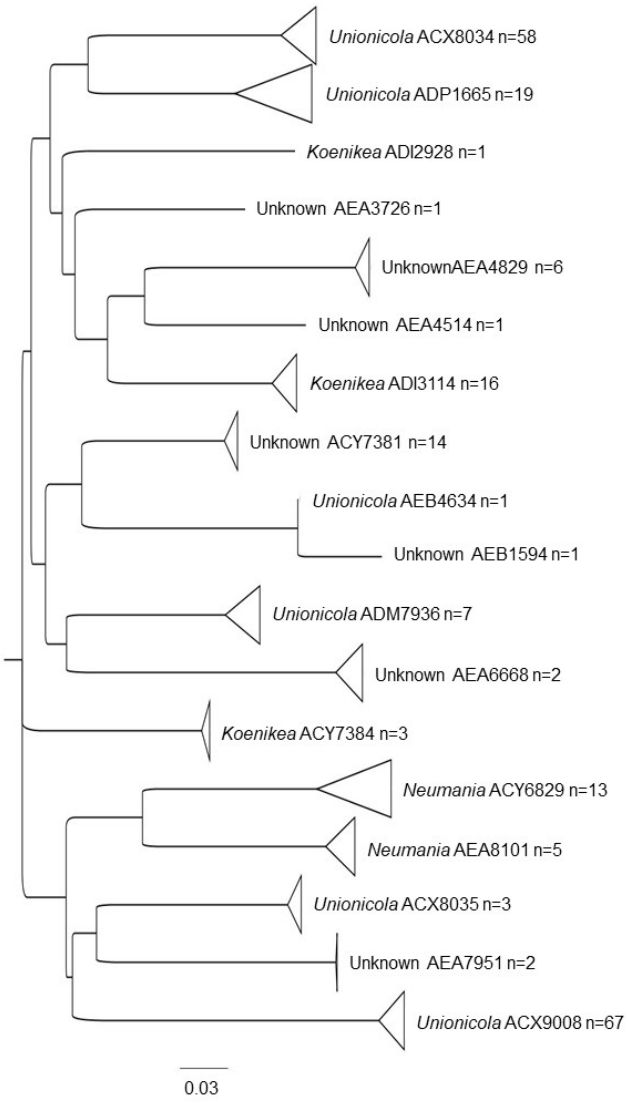

Figure 4. Neighbor Joining (NJ) tree for Unionicolidae family.

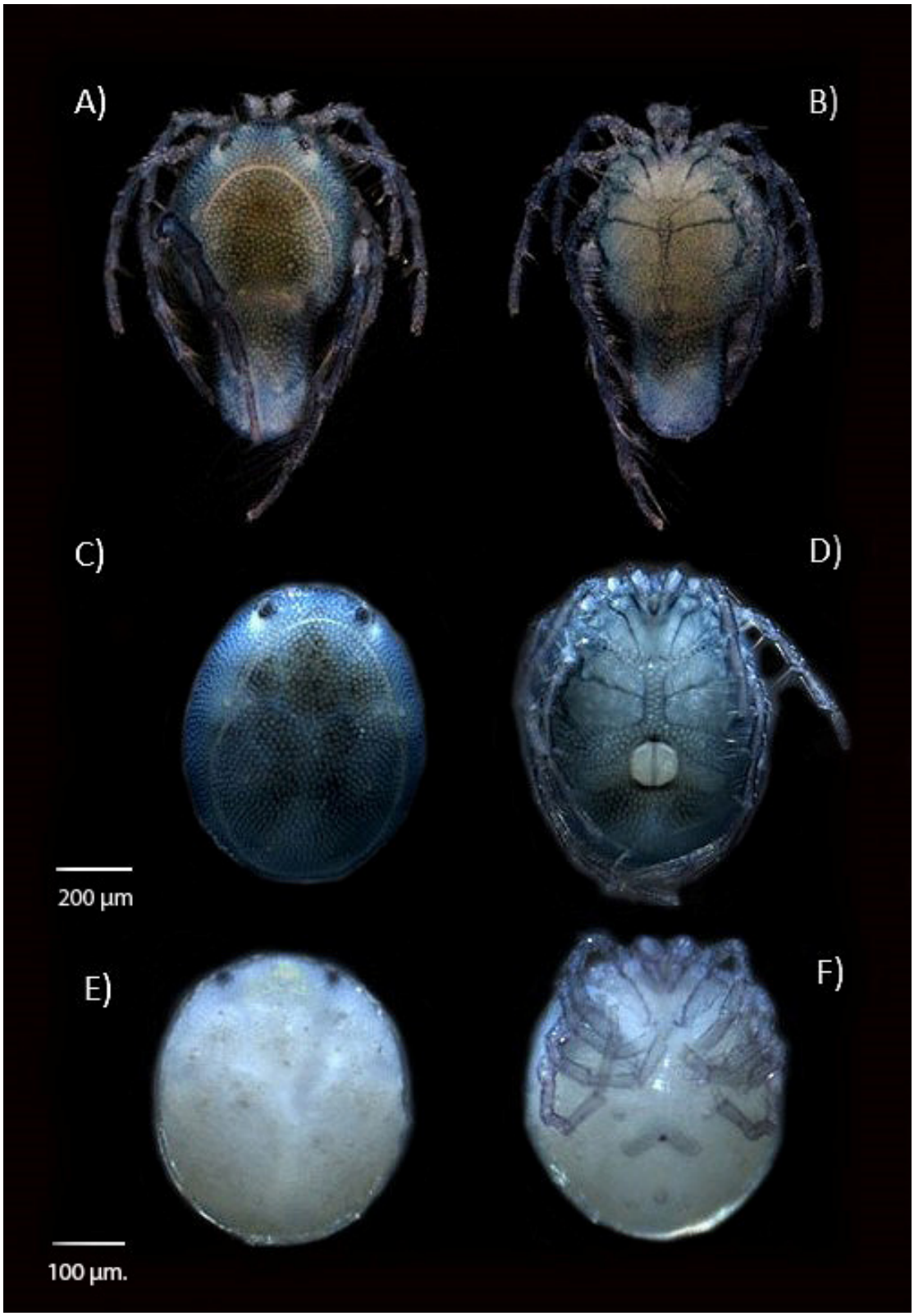

Figure 5. *Arrenurus* sp. BIN ACX8463: (**A**,**B**) dorsal and ventral view of male, (**C**,**D**) dorsal and ventral view of female, and (**E**,**F**) dorsal and ventral view of nymph.

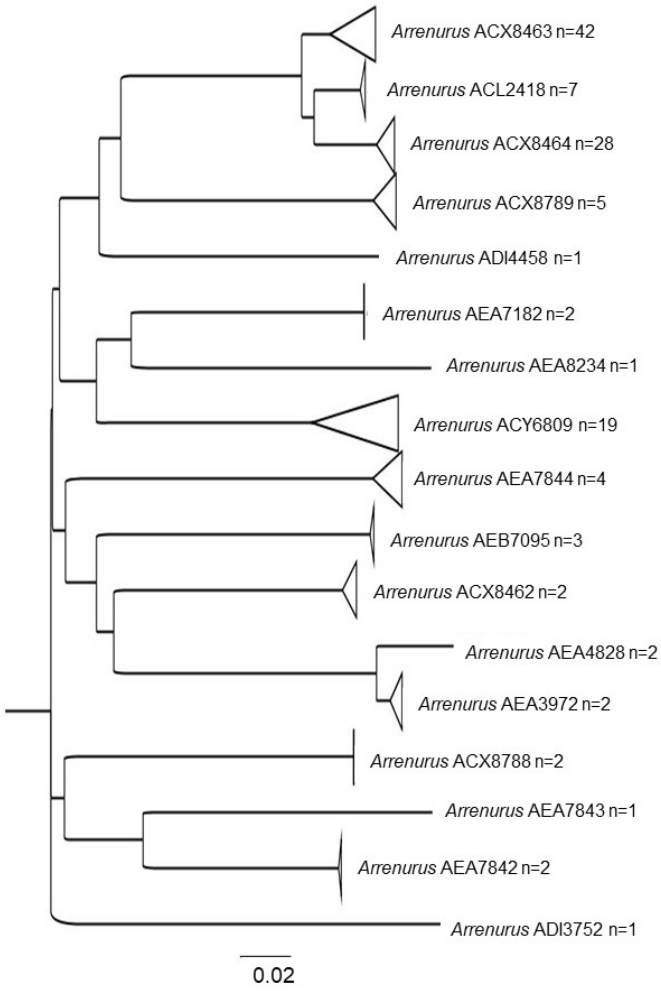

Figure 6. NJ tree for Arrenuridae family.

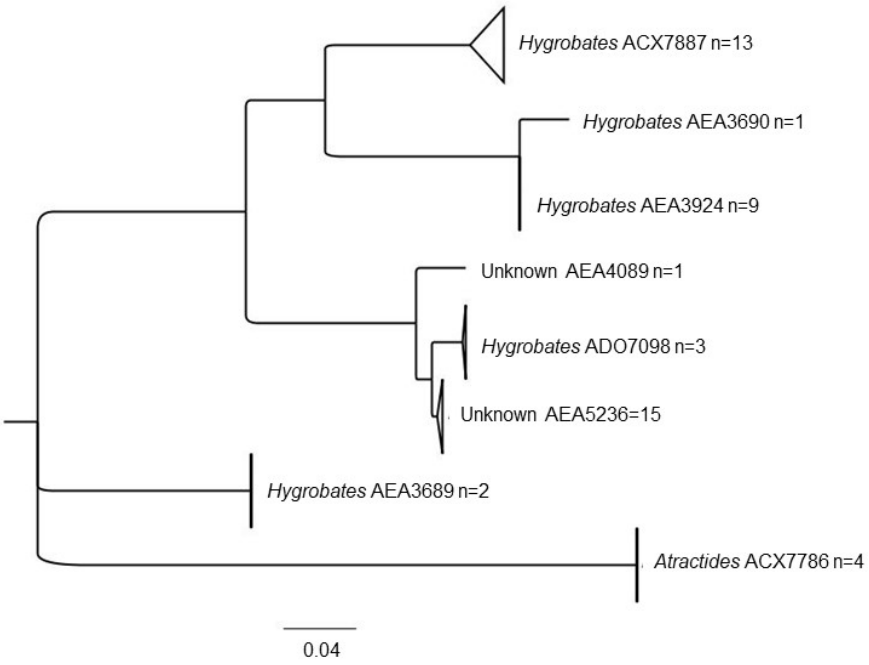

Figure 7. NJ tree for Hygrobatidae family.

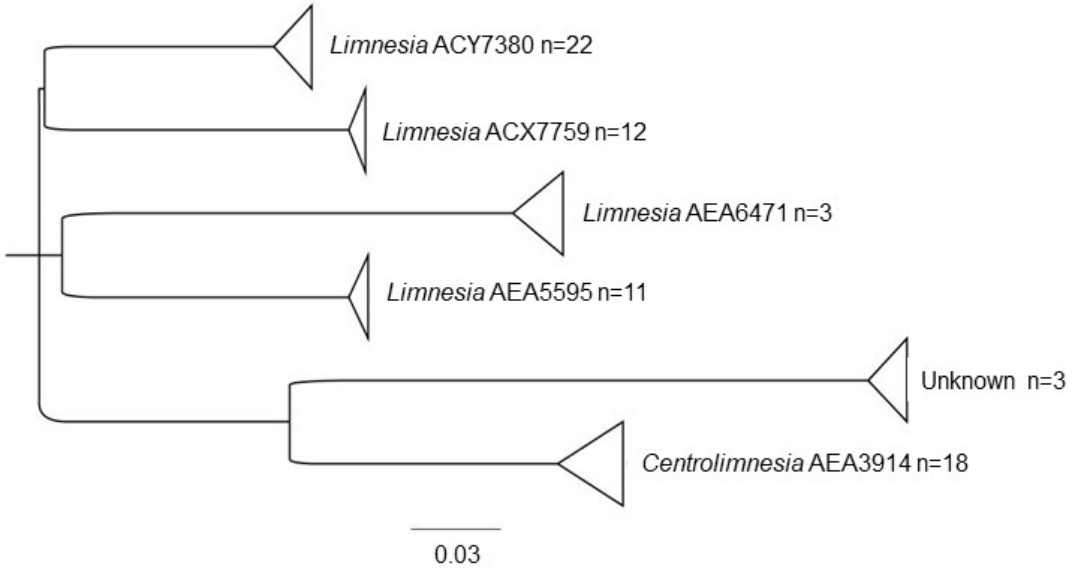

Figure 8. NJ tree for Limnesiidae family.

3.2. BIN Assemblies in the PY

From the total, 58 BINs were present in one or a maximum of three localities, possibly forming unique species assemblages (Tables 1 and 2). The Jaccard index value, in general, for all the localities, was zero or extremely low. However, some systems shared a percentage of their water mite fauna composition as follows: Chichancanab lagoon and Cenote El Padre (44%), Chichancanab lagoon and Cenote Km 48 (44%), Cenote El Toro and Cenote Santa Teresa (33%), Cenote Tres Reyes II and Cenote El Toro (33%), and Cenote Cocalitos and Cenote Escuela Normal (43%). The two latter are important because they are two different water systems inside the Bacalar Lagoon. Despite having such spatial relationship, each system seemed to have a different composition of water mites (Figures 9 and A1, Appendix A).

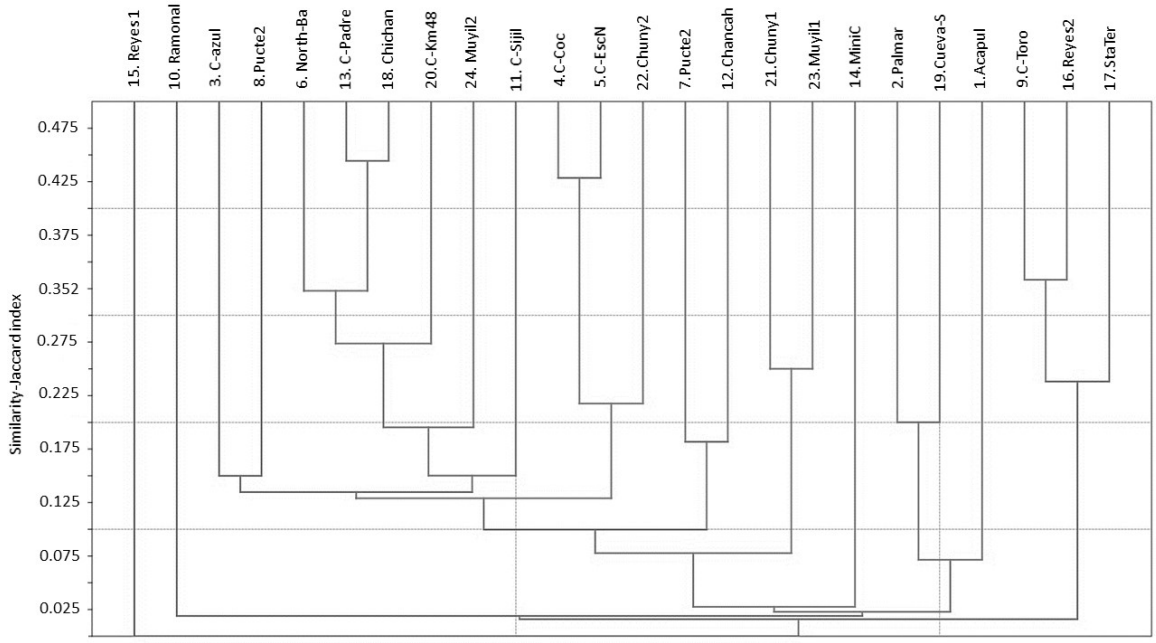

Figure 9. Similarity in water mites' composition between locations. Numbers in front of location name are the same as in Table 1.

4. Discussion

For the first time, a general analysis of the potential richness of water mite fauna in the central–southern part of the Yucatan Peninsula (Mexico), based on DNA barcodes was completed. Our results indicate an 11-fold increase in the number of species found previously in Quintana Roo state and twice the number of species registered in all the PY (in the three states, namely Campeche, Quintana Roo, and Yucatan) [6–8]. Out of 77 BINs, 58 are new in BOLD and seem to have a restricted distribution. This result indicates the presence of a unique set of environmental conditions and a particular water mite fauna composition of which most likely could be undescribed taxa. We need to study mite fauna in a wider geographic region to support this point; however, we have seen that most species are not widely distributed in our study area.

In the case of *Hydrodroma*, our results indicate the presence of only one morphospecies in eight sampling sites and has a proper correspondence with the unique BIN ADF3732. Previous research identified two species in the PY, *H. peregrina* Cook, 1980, and *H. despiciens* Marshall, 1936. However, for both species, Cook (1980) noticed distinctions from the type specimen. A recent study, using integrative taxonomy [30], compared sequences with the specimens collected from the Cenote Azul (Mexico) (*Hydrodroma* ADF3732), and the authors concluded that it was not *H. despiciens* [30] and probably not *H. peregrina*, due to the differences noticed by Cook [6]. Consequently, *Hydrodroma* BIN ADF3732 is probably a new species that needs to be formally described and is likely endemic to Southern Mexico.

Similarly, in the case of Unionicolidae, we registered 18 BINs (Figure 4 and Table 2). Previous records include ten species for the PY. Three of them correspond to descriptions of *Koenikea indistincta* Marshall, 1936; *Koenikea neopectinifera* Cook, 1980; and *Neumania cenotea* Marshall, 1936. All of them were apparently restricted to this region. The rest are described from other localities in Mexico or different regions. For example, *Unionicola gracilipalpis tenuis* Cook, 1980, was recorded in Campeche, Michigan, and Canada, but the type locality is in Haiti. Nevertheless, *U. gracilipalpis* was originally described from Europe. It is possible that this subspecies could be a full species, but we need to compare the type material to reach such a conclusion. *Unionicola (Pentatax) furculopsis* Cook, 1980, was described from Oaxaca state, but it was found in the Cenote Azul and Bacalar Lagoon by Otero-Colina [7]. Nevertheless, he noticed a similarity with *U. furcula* (Lundblad, 1935) and described some characteristics that the type species did not have, such as denticles in the gnathosoma base. These differences could be critical to identifying a different species, but more detailed research is required. *Neumania (Neumania) diversipalpa* Cook, 1980, was originally described from a single male in a river in Chiapas, based on an adult female, was recorded in the Cenote Azul by Otero-Colina [7]. The match male–female should be made from the same locality or at least after DNA barcodes have been obtained.

These are some examples of the taxonomic uncertainties that exist for water mites from the PY; however, our goal was not to discuss all previous identifications. These are just examples of the taxonomic impediment that still exists about "subspecies", "forms", and species recorded far away from the type locality or in an extremely different habitat from the original site. Some studies have revealed that species previously considered to be cosmopolitan are not really [30]. Many of them could be actually new species or species complexes. We consider that at least 15 OTUs of the Unionicolidae recognized by different BINs are possible new species.

Likewise, for Arrenuridae, there are seven species reported from the PY [8], six *Arrenurus* from the subgenus *Megaluracarus*, and one from the subgenus *Arrenurus*. Most of these reports are from Campeche and only one from Quintana Roo and Yucatan. We found 17 putative species of *Arrenurus* (Table 2 and Figure 6). Only three of these 17 BINs appear in multiple locations. The remaining 14 were found in only one or two close sampling sites (Table 2). After a comparison with the 135 BINs of arrenurids currently in BOLD, 94% of the BINs that we found appear juts in the south of Mexico. Other studies have previously documented the endemism of this family in other regions of the world [31–33]; however, this cannot be verified until a detailed morphological review of the specimens is made.

Another important achievement of this study is the pairing of males and females in nine BINs of this group that exhibit a high sexual dimorphism. Pairing the nymphal state in another three BINs will also allow us to make more complete formal descriptions if this species turns out to be undescribed (Figure 5).

The Hygrobatidae were the third richest group that we found (Table 2 and Figure 7). These are the first records for the PY. They were common in some locations (personal observation) that were previously surveyed [6,7]. This family seemed to be rare in the 1980s, when the previous studies took place. Some authors [2,34,35] suggest that several members of this family, *Hygrobates* included, are indicators of pollution and environmentally stressed water bodies. They were found in places like Cenote Cocalitos, Palmar, and Cenote Azul, with strong development of tourism (Tables 1 and 2). However, we must clarify the identity and habitat preferences of the species found in order to conclude if they indicate some level of environmental degradation. They may just be adapted to the extreme conditions of these places due to the presence of carbonates [10]. Nevertheless, previous surveys overlooked them.

The uniqueness of each aquatic system is clearly supported by the low values of the Jaccard index between the localities (Figure 9 and Appendix A Figure A1). For example, Cenote Azul and Cenote Cocalitos (Figures 1 and 2) are two localities with a distance of 160 m, but their similarity index is only 0.13 (Figure 9). This supports previous studies that found a difference in water quality and absence of communication between Cenote Azul and Bacalar [10]. Of the 14 BINS found in Cenote Azul and 21 found in Cocalitos, they only share four: *Limnochares* ACY6840, *Hydrodroma* ADF3732, *Unionicola* ACX8034, and *Arrenurus* ACX8463. These two systems have been extensively sampled, and their differences are also reflected in the composition of their planktonic communities [13,23]. Related studies have found that water mite assemblages are partially explained by environmental parameters such as temperature, conductivity, or pH and can almost be predicted by the potential prey groups, mainly cladocerans, copepods, and chironomids [36].

The PY ecosystems are characterized for being a mosaic of multiple habitats, with extreme differences in hydrogeochemistry conditions [9,10,12]. Their unique configuration that is structured after faults, underground and surface intermittent connections, and sinkholes (the most common surface water systems) suggests that they could be isolated. Therefore, they exhibit a distinctive diversity. Additionally, the distribution of water mites is known to be influenced by the substrate, type of vegetation, water flow, and depth. For example, El Palmar and Acapulquito present microhabitats with slow flow current combined with pools and submerged vegetation. As a result, we found a mixture of taxa with lotic environment preferences as *Torrenticola* and species with lentic preferences as *Arrenurus* or *Unionicola* [2].

Evidently there are still several unanswered questions in terms of the diversity of water mites in the PY. For example, are there specific assemblies for microhabitats? What causes differences in abundance between species? What are the phylogenetic relationships between them, or how is their evolutive history in the PY? Finally, we consider this analysis as a preliminary step toward the formal description of all the species that we found, including morphological details of the vouchers, in order to assign them a Linnaean name; once this step has been carried out, many of our hypotheses about restricted distributions and new endemic species could be fully tested.

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Appendix A

	AC	PAL	CAZ	COC	CEN	BAN	CP1	CP2	CT	RAM	CSN	CCV	CP	MIC	CR1	CR2	CST	CHI	CS	K48	CH1	CH2	MU1	MU2
AC	-																							
PAL	0.14	-																						
CAZ	0.00	0.06	-																					
COC	0.03	0.08	0.13	-																				
CEN	0.00	0.08	0.05	0.43	-																			
BAN	0.00	0.12	0.22	0.35	0.15	-																		
CP1	0.00	0.00	0.10	0.15	0.13	0.15	-																	
CP2	0.00	0.08	0.15	0.11	0.13	0.10	0.06	-																
CT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	-															
RAM	0.00	0.00	0.05	0.00	0.00	0.05	0.06	0.00	0.00	-														
CSN	0.00	0.00	0.06	0.09	0.09	0.06	0.00	0.09	0.00	0.00	-													
CCV	0.00	0.00	0.06	0.09	0.00	0.20	0.18	0.00	0.00	0.00	0.17	-												
CP	0.00	0.00	0.11	0.17	0.15	0.33	0.15	0.07	0.00	0.07	0.29	0.43	-											
MIC	0.00	0.00	0.07	0.05	0.10	0.00	0.10	0.10	0.00	0.00	0.00	0.00	0.00	-										
CR1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-									
CR2	0.00	0.00	0.00	0.04	0.07	0.05	0.00	0.15	0.33	0.00	0.00	0.00	0.09	0.00	0.00	-								
CST	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.14	-							
CHI	0.00	0.09	0.24	0.12	0.14	0.31	0.07	0.23	0.00	0.07	0.11	0.10	0.44	0.00	0.00	0.08	0.00	-						
CS	0.00	0.20	0.00	0.05	0.11	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-					
K48	0.00	0.10	0.11	0.13	0.15	0.18	0.15	0.25	0.00	0.07	0.13	0.00	0.20	0.00	0.00	0.09	0.00	0.44	0.00	-				
CH1	0.00	0.00	0.13	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-			
CH2	0.00	0.14	0.09	0.19	0.25	0.14	0.11	0.18	0.00	0.00	0.08	0.00	0.06	0.00	0.00	0.06	0.00	0.13	0.09	0.13	0.17	-		
MU1	0.00	0.00	0.17	0.12	0.00	0.17	0.07	0.07	0.00	0.00	0.11	0.22	0.18	0.00	0.00	0.00	0.00	0.08	0.00	0.08	0.25	0.20	-	
MU2	0.00	0.00	0.06	0.09	0.18	0.06	0.08	0.08	0.00	0.00	0.17	0.00	0.25	0.00	0.00	0.11	0.00	0.22	0.00	0.25	0.17	0.07	0.00	-

Figure A1. Matrix of Jaccard index values for all pairs of sampled locations, based on water mite BINs. AC = Acapulquito, PAL = Palmar, CAZ = Cenote Azul, COC = Cenote Cocalitos, CEN = Cenote Escuela Normal, BAN = Bacalar Norte, CP1 = Cenote Pucte 1, CP2 = Cenote Pucte 2, CT = Cenote El Toro, RAM = Ramonal, CSN = Cenote Sijil Noh Ha, CCV = Cenote Chancah Veracruz, CP = Cenote del Padre, MIC = Minicetonte, CR1 = Cenote Tres Reyes 1, CR2 = Cenote Tres Reyes 2, CST = Cenote Santa Teresa, CHI = Chichancanab, CS = Cueva de las serpientes, K48 = Cenote Km.48, CH1 = Chunyaxche 1, CH2 = Chunyaxche 2, MU1 = Muyil 1, and MU2 = Muyil 2.

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