

## Article

# Phenetic and Genetic Variability of Continental and Island Populations of the Freshwater Copepod *Mastigodiaptomus ha* Cervantes, 2020 (Copepoda): A Case of Dispersal?

Adrián Cervantes-Martínez <sup>1,\*</sup>, Martha Angélica Gutiérrez-Aguirre <sup>1</sup>, Eduardo Suárez-Morales <sup>2</sup> and Sarahi Jaime <sup>1</sup>

<sup>1</sup> Unidad Académica Cozumel, Universidad de Quintana Roo, Av. Andrés Quintana Roo, Calle 11 con calle 110 sur s/n, C.P. 77600 Cozumel, Mexico; margutierrez@uqroo.edu.mx (M.A.G.-A.); 1518305@uqroo.mx (S.J.)

<sup>2</sup> Unidad Académica Chetumal, El Colegio de la Frontera Sur, Av. Centenario km 5.5, C.P. 77014 Chetumal, Mexico; esuarez@ecosur.mx

\* Correspondence: adcervantes@uqroo.edu.mx

**Abstract:** The diversity of freshwater zooplankton is still little known in Mexico, particularly in reference to insular zooplankton communities. Diaptomid copepods (Crustacea: Copepoda: Calanoida) are a widespread group worldwide, and Mexico harbours high diaptomid diversity. Based on a recent sampling of freshwater zooplankton on a Caribbean Island of Mexico, we present the first record of a diaptomid copepod from an island freshwater ecosystem. It shows the well-known tendency of Neotropical diaptomids to have restricted distributional patterns and high levels of endemism. The species recorded, *Mastigodiaptomus ha* (Cervantes-Martínez, 2020) appears to have a restricted distribution in the Yucatan Peninsula (YP), and the island as well. In order to explore potential differences between the island and continental populations of this species, its phenetic and genetic diversity was analysed by performing morphological comparisons and also by exploring differences of the habitat conditions and genetic sequences (CO1 gene). Our analysis revealed a low (average = 0.33%) genetic divergence between both populations; likewise, both the morphology and habitat conditions closely resemble each other in these two populations. The low genetic divergence between the continental and island populations of *M. ha* suggests an early common origin of the species in the geological history of the YP.

**Keywords:** barcoding; Calanoida; diaptomids; freshwater; insular water bodies; new record



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## 1. Introduction

The diverse zooplankton community inhabiting the epicontinental and underground freshwater ecosystems of the Yucatan Peninsula (YP) can be largely constituted by calanoid copepods belonging to the most successful freshwater group; the family Diaptomidae. Diaptomids tend to have restricted distributional patterns, with many endemic species in the Neotropical region [1].

*Mastigodiaptomus* is one of the most diverse genera in Mexico, currently including 13 species. The genus is widely distributed in the Neotropical region, including the Caribbean islands, Central America, and areas of the Southern United States [2,3].

Recently, Gutiérrez-Aguirre et al. [3] described three new species of the genus from Mexico; *Mastigodiaptomus cihuatlan* (Gutiérrez-Aguirre, 2020), *M. alexei* (Elías Gutiérrez, 2020), and *M. ha* (Cervantes-Martínez, 2020). The latter was found in sinkholes (locally known as cenotes) in the northeastern continental zone region of the YP.

After 15 years of basic studies on the freshwater and anchialine zooplankton in Cozumel Island [4–6], this is the first report of a diaptomid copepod on a Mexican island. Previously, *M. ha* has been recorded in continental freshwater systems in the north-northeastern region of the YP [3]. In this study we analysed the phenetic and genetic distances between the island and continental populations, and specimens from the type

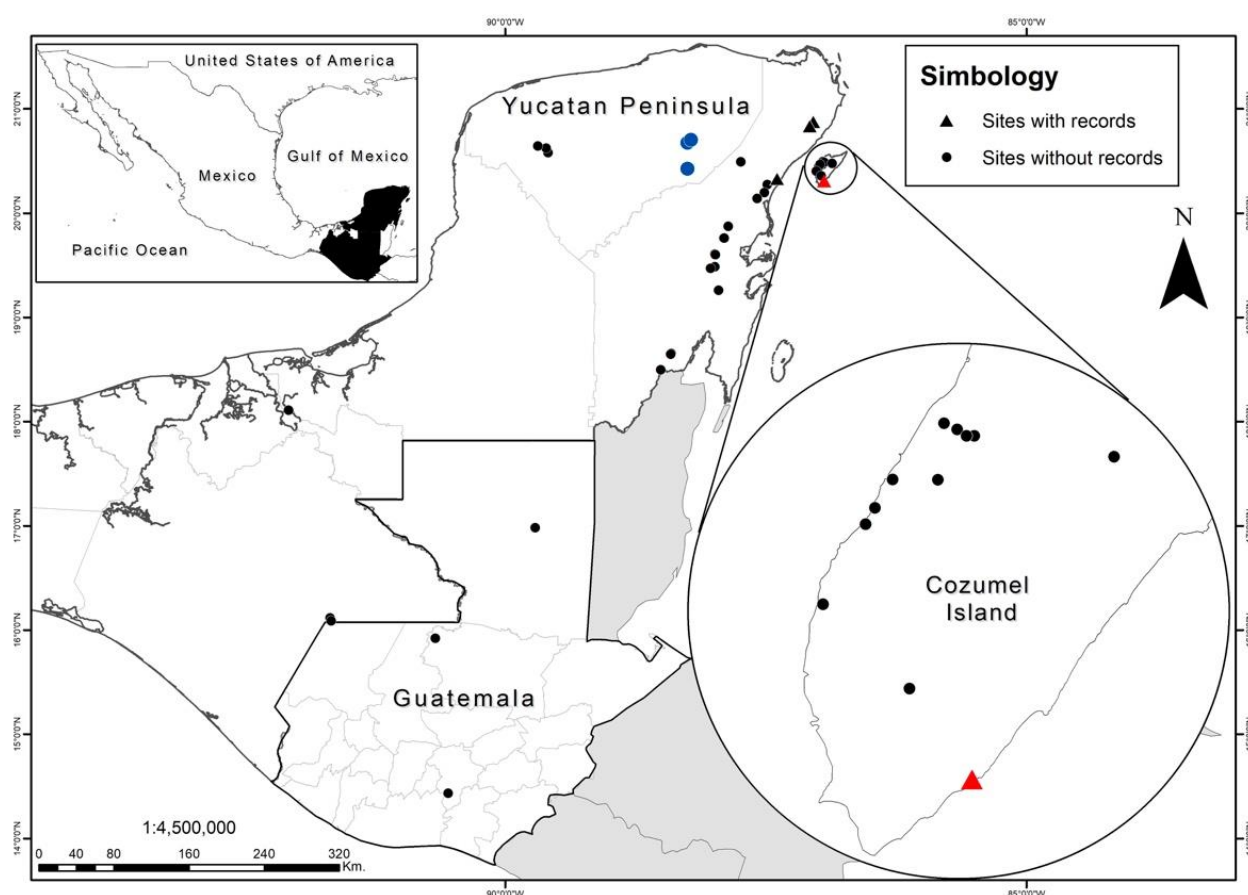
locality of *M. ha*, adding new molecular barcodes revealing that, despite the fact that these two populations were isolated for over 8000–6000 years, they are phenotypically and genetically similar.

This work confirms that YP copepod fauna provides the best-known Mexican region for harbouring the greatest diversity of the *Mastigodiatomus* species in Central America and the Caribbean region [2,3,7].

## 2. Materials and Methods

### 2.1. Study Sites and Sampling Methods

Cozumel Island is located off the northeastern coast of the YP, in the northwestern region of the Caribbean Sea (Figure 1) and, because of its area (~500 km<sup>2</sup>), it is the third largest island in Mexico and the most populated. An 18 km wide channel separates it from the continental YP.



**Figure 1.** Collection sites for zooplankton samples in the YP, Mexico (2005–2020), where diaptomids are present. Black circles are negative records of *Mastigodiatomus ha*; triangles are positive sites for records of *M. ha*. The red triangle is the place where the species was founded on Cozumel Island. Blue circles indicate probable presence of this species.

The YP is considered one of the largest karstic aquifers in Mexico and the world [8]; a system that includes Cozumel Island, which was once part of the continental YP geologic plate of the YP (~25 Mya). Like all karstic aquifers, underground aquatic systems are extended (freshwater or anchialine); sinkholes and some superficial lagoons are the surface features of karstic environments [9,10]. Cozumel island has some similar aquatic systems to those mentioned before, but they differ in area, size, and depth [11,12].

### 2.2. Zooplankton Analysis

During the last 15 years, a systematic sampling of freshwater and anchialine zooplankton has been performed on the YP and Cozumel Island (Figure 1), with high zooplankton

diversity and crustacean endemism [4–6,13]. Water samples for biotic and abiotic variables were collected on the southern part of the island, after the atypical influence of tropical storms in September–October 2020 on Cozumel Island [14].

The species classification of Copepoda was performed according to the methods outlined by Elías-Gutiérrez et al. [15] and Suárez-Morales et al. [16], several specimens were compared with the recently described *M. ha*, inventoried in the north-northeastern region of the YP continental plate [3].

Then, the phenetic variability between the continental vs. island populations of *M. ha* was determined, considering the morphological, environmental, and geological features of the locations inhabited by the copepods. Chemical environmental variables, related to the ionic content, and temperatures were measured in situ with a professional-plus YS Datasonde® (Xylem Inc., Yellow Springs, OH, USA). The trophic status of these systems was determined by the chlorophyll *a* concentration [9]. The sequence of the COI gene was used as evidence of the genetic variability between continental and insular populations of *M. ha*.

### 2.2.1. Continental Populations Analysed

1. Adult female holotype, dissected on semi-permanent slide (ECOCH-Z-10319), adult male allotype, dissected, mounted on semi-permanent slide (ECOCH-Z-10320), and 20 adult females and 20 males preserved in 96% ethanol and one drop of glycerine (ECOCH-Z-10321). Cenote 7 Bocas, Quintana Roo, Mexico, 20°52'36" N; 87°02'37" W, the type locality of *Mastigodiaptomus ha*.

2. Specimens obtained from Verde Lucero ( $n = 30$ , ECOCH-Z-10327; 20°52'09.57" N, 87°04'37.52" W) and Boca del Puma ( $n = 6$ , ECOCH-Z-10326; 20°52'179" N, 87°03'18" W) in Quintana Roo, Mexico were also analysed.

### 2.2.2. Cozumel Island Population

Twenty-two adult females and 36 adult males from 25 Horas Lagoon (20°18'39.7" N, 86°56'14.2" W) (ECO-CH-Z-10539). The acronym ECOCH-CH-Z refers to the Zooplankton Reference Collection held at El Colegio de la Frontera Sur, Chetumal, Mexico.

The morphological variability between the two groups of populations was analysed with dissected and whole specimens using a compound Nikon Eclipse 50i microscope. Light microscopic images of variable features were captured with a Lumenera Infinity1 Y-IDT camera (Teledyne Lumenera, Ottawa, Canada) and arranged in Adobe Photoshop V. 6.0 following the current taxonomic standards for the genus *Mastigodiaptomus* [2,17,18].

The DNA extractions of the COI gene, PCR products, and sequence alignment between populations were conducted following the methods of Ivanova et al. [19], Hebert et al. [20], Prosser et al. [21], and Elías-Gutiérrez et al. [22], in accordance with the protocols of the Barcoding Laboratory of Life (ECOSUR, Chetumal, Mexico). Two specimens from the type area, three from the Verde Lucero, and two from the Boca del Puma were considered the continental populations in the genetic analysis, and 28 specimens from the Cozumel population were all included.

Cluster analyses of these sequences were performed to obtain a graphic representation of the divergences among the specimens by using Molecular Evolutionary Genetics Analysis (MEGA X) software (MEGA Freeware, University of Pennsylvania, Philadelphia, PA, USA).

Sequences were aligned to 600 base pairs (pb) with CLUSTAL W, and the Kimura 2 parameter (K2P) distance model was used to calculate the sequence divergences. Neighbour-joining (NJ) clusters were created with the gamma distribution model.

All sequences > 500 pb were added to the public data dataset named *Mastigodiaptomus*, created in the Barcode of Life Data Systems portal (BOLD SYSTEMS, <http://boldsystems.org/index.php>, accessed on 15 April 2021). In the dataset DOI: 10.5883/DS-MMASTIGO (The Barcode of Life Data System, University of Guelph, Guelph, Canada, accessed on

15 April 2021), the individual sequences, trace files, collection data, and primer details are available.

### 3. Results

#### 3.1. Variability of Environmental, Genetic and Morphological Features

*Mastigodiaptomus ha* is a freshwater, free-living diaptomid copepod, that is apparently endemic to the north and northeastern zones of the YP. The aquatic ecosystems inhabited by this species showed the following environmental features: low elevations ( $13.2 \pm 6.0$  masl), oligo-mesotrophic conditions ( $\leq 0.70$  mg/m<sup>3</sup> of chlorophyll *a*), low oxygen concentrations ( $1.5 \pm 1.8$  mg/L), tropical climates ( $26.8 \pm 1.9$  °C), freshwater ( $0.7 \pm 0.2$  ppt), low conductivities ( $1396 \pm 559.2$  µS/cm<sup>3</sup>) (Table 1), and physically the sinkholes where *M. ha* was recorded are type 2, 3, and 4 sinkholes according to Hall [23] (Table 1).

**Table 1.** Limnological characteristics of the aquatic habitats inhabited by *Mastigodiaptomus ha* (Quintana Roo, Mexico). Elev = elevation (masl), T = water temperature, O<sub>2</sub> = dissolved oxygen (mg/L), EC= electrical conductivity (µS/cm<sup>3</sup>), Sal = salinity (ppt), TS = trophic state.

Place Name	Coordinates	Sinkhole Classification [23]	Elev	T	O <sub>2</sub>	EC	Sal	TS
Chemuyil	20°21'38.7" N 87°23'98.1" W	Vertical walls with wide aperture (as a glass)	10	29.2	4.6	2274	1.1	Oligotrophic
Verde Lucero	20°52'08.7" N 87°04'37.5" W	Vertical walls with wide aperture (as a glass)	18	24.7	1.8	1414	0.8	Mesotrophic
7 Bocas	20°52'35.8" N 87°02'37.5" W	Cavern, with lateral entrance leading to a chamber with water	16	25.6	0.3	1365	0.7	Oligotrophic
Boca del Puma	20°52'17.9" N 87°03'18" W	Cavern, with lateral entrance leading to a chamber with water	18	26.3	0.7	1170	0.6	Oligotrophic
25 Horas	20°18'39.7" N 86°56'14.2" W	Superficial lagoon ("aguada")	4	28.6	0.2	742	0.4	Oligotrophic

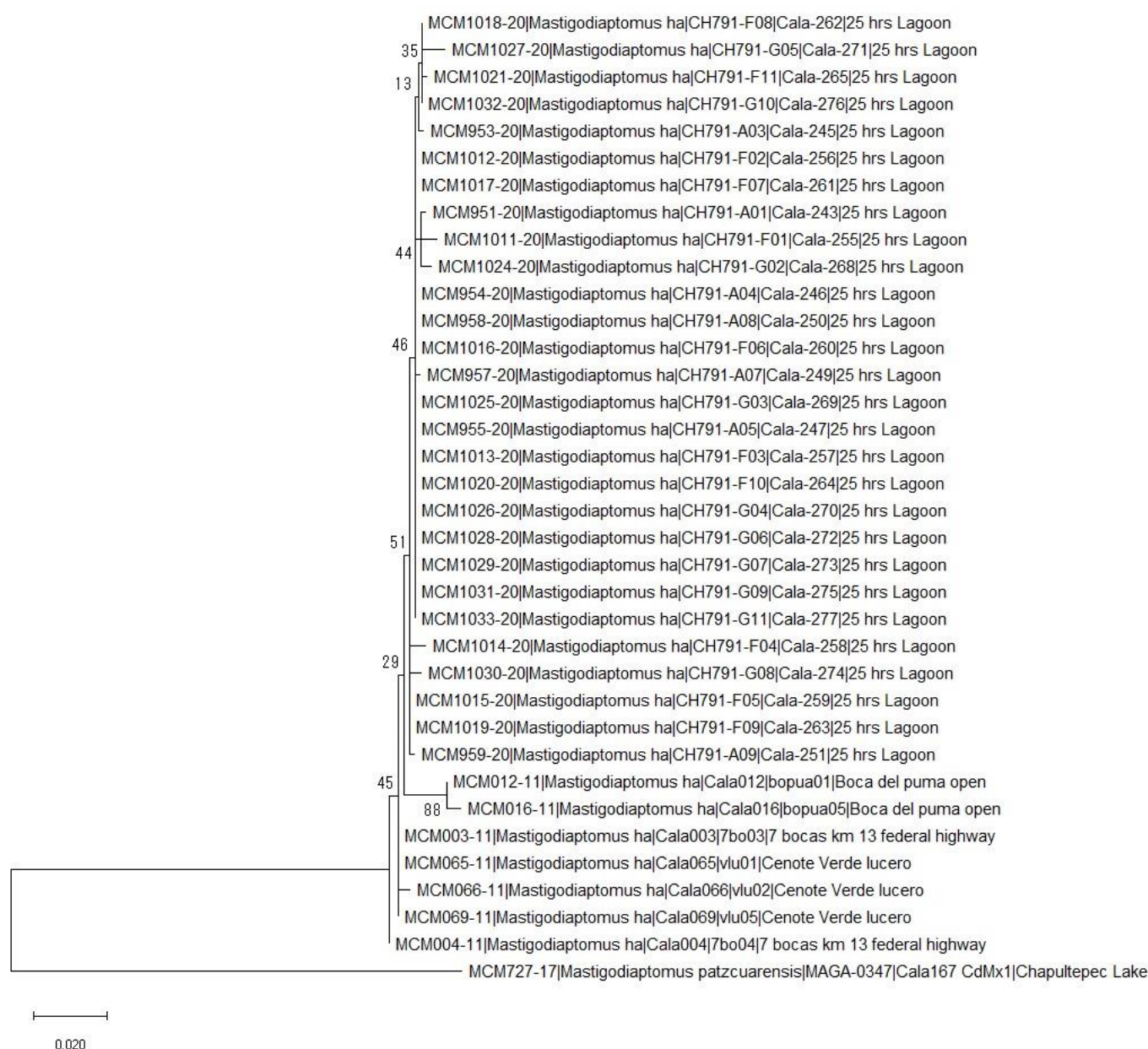
This kind of aquatic ecosystem is common on the northern and the eastern fringe zones of the YP, where the most recent, highly permeable sediments (i.e., Miocene, Pliocene, and Quaternary) are widespread [8,24]. These regions are therefore dominated by underground currents or superficial lagoons that formed recently in geological time [8].

#### 3.2. Genetic Variability

The consensus tree with the highest log likelihood is shown in Figure 2. The bootstrap percentages of trees, in which the associated taxa were clustered together, are shown next to the branches. The extended dendrogram displays that the four continental populations and one island population analysed showed one group with low genetic divergence: the BOLDsystems generated the BIN AAU1038 (The Barcode of Life Data System, University of Guelph, Guelph, Canada, accessed on 15 April 2021). The genetic divergence between populations was 0.33% on average (Table 2).

**Table 2.** Sequence divergence distribution at each taxonomic level examined. To avoid confusion, in this analysis continental vs. island specimens were labelled as two different species, *Mastigodiaptomus ha* vs. *Mastigodiaptomus* sp. (= *M. ha*-island population), to perform a comparison between the populations.

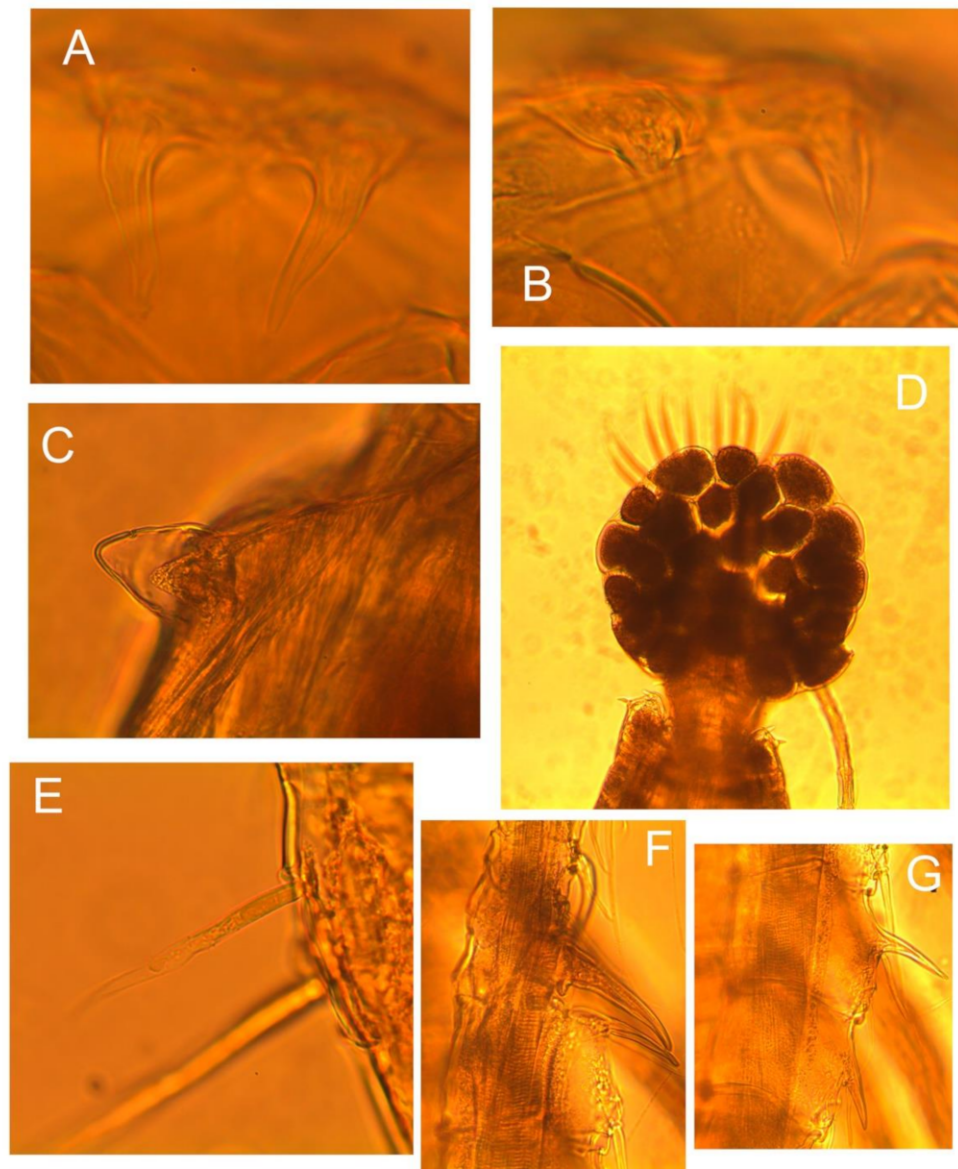
Label	N	Taxa	Comparisons	Min Dist.	Mean Dist.	Max Dist.	SE Dist.
Within species	36	2	416	0.00	0.33	2.22	0.00
Within genus	37	1	179	0.34	4.64	20.15	0.04



**Figure 2.** Analysis for *Mastigodiptomus ha* (Quintana Roo, Mexico) with the maximum likelihood method to observe genetic relationships between continental and insular populations (1000 replicates), based on the COI gene. The scale length represents the percentage of genetic distance between branches.

The body sizes of specimens of the island population of *M. ha* were larger than those of the continental population specimens; in the continental populations, the total body lengths were similar between females and males, ranging from 1.2 to 1.3 mm; whereas the females of Cozumel island are larger (i.e., 1.6 to 1.78 mm,  $n = 28$ ); males are also larger in the island population (1.45 to 1.5 mm,  $n = 35$ ).

The rostral points shape was also variable in all the analysed continental and island populations: short with rounded points, medium-sized with rounded points or even, large and acute in females (Figure 3A–B). Whereas only 20% of the continental females bear a dorsal keel-like process on the fifth prosomite, all the examined females of the island population (~60 specimens) possess one dorsal, triangle-shaped process (Figure 3C). Twenty percent of the surveyed island females bear large egg sacs with 23–30 eggs (Figure 3D), whereas continental females did not bear egg sacs.



**Figure 3.** *Mastigodiaptomus ha*, adult female from 25 Horas Lagoon (island population): (A) large curved rostral spines; *M. ha*, adult female from Verde Lucero Lagoon (continental population): (B) short acute rostral spines; *M. ha*, adult female from 25 Horas Lagoon: (C) dorsal process, lateral view; (D) egg sac, dorsal view; (E) modified setae on antennular segment 11. *M. ha*, adult male from 25 Horas Lagoon: (F) right antennule segments 13, 14; (G) same, segments 15, 16.

In *M. ha*, the presence of modified setae on both female and male antennules was notable as a distinctive feature; these setae are large, flattened, and distally expanded (Figure 3E). In the island population, the spines of male right antennular segments 13–16 are proportionally larger than those of all the continental populations (Figure 3F–G).

#### 4. Discussion

After systematic surveys of freshwater and anchialine zooplankton were conducted for approximately 15 years in the YP, including on Cozumel Island, this is the first finding on record of a freshwater, free-living calanoid diaptomid on a Mexican island. In the southern region of Cozumel Island where *M. ha* was collected, the pools are flooded in the rainy period (July to October) and the 25 Horas Lagoon suffers a reduced catchment area during the dry season (March to May), with a proportional decrease in the copepod's population.

Therefore, it is hypothesised that *M. ha* is an endemic form of some freshwater systems located in the north-northeastern regions of the YP, and is probably present on other Caribbean islands with geological histories that are homologous to that of Cozumel Island.

The physiography of the YP has been influenced by successive marine regression and transgression processes: during the Cretaceous Period (~145 Mya) and the Pleistocene Yarmouth interglacial stage (220–170 Kya, during which the sea level rose 30 m above the current level), the YP was submerged [7,25]. The YP and Cozumel have been separate since the early Cenozoic Era (~65 MYa) [26]. Allopatric speciation has been advanced as a possible explanation for the divergence of two species of anchialine remipedes: *Xibalbanus cozumelensis* (island species) and *X. tulumensis* (continental). A weak gene flow between these two populations was deemed probable because the marine regressions likely gave place to intermittent communication between these populations, which is not the case for epigean freshwater crustaceans.

The eastern coast of the YP remained submerged for about 33 MY, between the Paleocene and Oligocene periods. During these periods of marine regression, freshwater forms could have recolonised the emerged lands. According to Suárez-Morales [7], one of these forms was an ancestor of the genus *Mastigodiptomus*, probably related to *M. albuquerquensis*, currently deemed to represent a species complex. This ancestral form successfully radiated across the Yucatan Peninsula, including its central core and eastern coast [7]. The occurrence records of *M. ha* more or less seem to fit the area flooded by seas in the Pleistocene interglacial period, which suggests that this freshwater species could be relatively young (<220–170 Kya).

The most recent soils on the coastal fringe of the peninsula were formed during the Pleistocene Epoch (~1.8 Mya) and the Holocene Epoch (11 Kya) with predominant calcites and dolomites as the most common mineral (of organic origin) [27]. The current physiography of the YP (including its islands and its complex and extensive hypogean landscapes) was present approximately 8–6 Kya [7,27,28]. The specific natures (freshwater, anchialine, or strictly marine) of the cavernous ecosystems in the region were influenced by the intermittent drought/cold periods that took place between 8 and 7, and 6 and 5 Kya [7,28], called stadial and interstadial periods during the Holocene period (~9 Kya). These dry and cold conditions in the YP during the Holocene period [29], the high reproductive rates in copepods, and phoresis of micro-crustaceans by birds [30], probably represented adequate conditions promoting the dispersal of epigean freshwater copepods through glacial refuges in emerged (probably cold lands), including the presence of *M. ha* in Cozumel Island and the eastern coast of the YP.

Additionally, the north and northeastern zones of the YP are considered to have low elevational contrasts, with plains lower than 20 masl, and these regions are apparently separated from the geologically older Meridional Peninsula by hills with elevation levels of 50 to 300 masl [31]. The groundwater ecosystems that span these plains are home to the greatest diversity of species and the highest number of endemic crustaceans in the YP [32]. In accordance with the distribution of other freshwater fauna, such as fish [33], the distribution of freshwater crustaceans that inhabit the plain region can apparently be explained by these geographic barriers.

The presence of *M. ha* individuals in this region supports the hypothesis that the YP is the area with the greatest known diversity and with the highest endemism of the *Mastigodiptomus* species in the Neotropical region, probably resulting from its habitat diversity and complex geologic history [2,7]. In the YP, seven diaptomid species have been recorded: *Arctodiptomus dorsalis* (Marsh, 1907); *Leptodiptomus novamexicanus* (Herrick 1895); *M. nesus* (Bowman, 1986); *M. reidae* (Suárez-Morales and Elías-Gutiérrez, 2000); *M. maya* (Suárez-Morales and Elías-Gutiérrez, 2000); *M. siankaanensis* (Mercado-Salas, Khodami, Kihara, Elías-Gutiérrez and Martínez-Arbizu, 2018), and *M. ha* (Cervantes-Martínez, 2020). The latter four are considered YP endemics [3,7,34]. The late Eocene marine regression exposed most of the YP [7]. This combination of geological, climatological, and

ecological conditions probably promoted the isolation conditions for local crustacean speciation and its current distributional patterns in the area [7].

Because low genetic divergence was revealed in and between the analysed continental and island populations of *M. ha*, it is hypothesised that the island population did not result from a very recent invasion process by humans, who have occupied the Cozumel island since 300 BC [35]. Instead, it is suggested that both the continental and island populations have an early common origin, and these populations were isolated after the Pleistocene Yarmouth interglacial stage; the slight morphological divergences featured, found within and between the examined populations of *M. ha*, support the previous statement, as well as a lack of evidence that the distribution of the *Mastigodiaptomus* species is promoted through resistance (or diapause) structures.

The genetic divergence found between populations was remarkably low, ranging between 0 and 2.22%: among the *Mastigodiaptomus* species, the greatest differences recorded within populations reach 2.76% on average [2]. The relatively low genetic, inter- and intra-population divergences found in *M. ha* might indicate the young age of the species. These low divergences have been found in freshwater fish whose current distributional ranges were glaciated during the Pleistocene period in America [36], and are probably related to the reduced population size in glacial refuges causing bottlenecks [37].

Morphological variabilities were described among and within the continental populations of *M. ha* in their original description [3]. In this study, when the continental populations were compared with the island population, a morphological variation was observed in body size (with striking differences between females and males), the rostrum shape, and the size of the spiniform process of the male right antennular segments 13–16.

In addition, the presence/absence of a dorsal process in females is a variable feature between and within populations of *Mastigodiaptomus texensis*, *M. amatitlanensis*, *M. albuquerqueensis*, *M. montezumae*, and *M. ha* [38]. We speculate here that the variability of this character appears to be related to the presence of fish co-occurring with *Mastigodiaptomus*: the dorsal process is absent or very reduced in presence of predator fish, but mostly present and well-developed when fish predation is absent or negligible (unpublished data).

This is the third report of a *Mastigodiaptomus* species inhabiting Caribbean islands with an elevation below 100 masl: *M. nesus* was recorded on San Salvador Island, the Cayman Islands, the Bahamas, and Cuba; *M. purpureus* in Cuba [39], and *M. ha* in Cozumel (this report). Five species of this genus have been recorded at altitudes  $\geq 1000$  masl: *M. montezumae* [40], *M. patzcuarensis*, *M. albuquerqueensis* [3,41], *M. suarezmoralesi* [38], and *M. amatitlanensis* [3]. It is likely that the ability of diaptomid genera to dwell in wide elevation and latitudinal ranges is a factor promoting the high regional diversity of *Mastigodiaptomus*.

Individuals of *Mastigodiaptomus* that inhabit island populations appear to be larger on average than their continental counterparts; for instance *M. purpureus*, exclusively recorded in Cuba, is one of the largest species (females = 2.5 mm long, males = 2.2 mm) [42,43]. The length of *M. nesus* in Cuban populations is 1.48 mm in females, and 1.34 in males [39] vs. 0.9–1.0 mm in both sexes in the continental populations [3,44]; the length of *M. ha* from Cozumel is 1.73 mm in females and 1.5 mm in males vs. 1.2–1.3 mm in both sexes in the YP continental populations [3]. As discussed above, it is likely that the presence of fish is related to decreasing copepod size, because in all mentioned cases in which larger island specimens occur, fish are absent.

## 5. Conclusions

After almost two decades of basic study on freshwater and anchialine zooplankton on Cozumel Island, this is the first report on *Mastigodiaptomus ha* (Cervantes-Martínez, 2020), a freshwater, free-living diaptomid copepod on a Mexican island.

This copepod was previously recorded in freshwater continental ecosystems on the north and northeastern region of the continental plate of the YP; this region is historically

and geologically similar to Cozumel Island, and slight morphologic and genetic differences were found between the continental and the island populations of *M. ha*.

Because low genetic divergences were observed between the analysed continental and island *M. ha* populations, both populations of the species probably resulted from the same founder effect.

The YP is the region in which the greatest number of copepod inventories has been documented and the greatest known diversity of the *Mastigodiaptomus* species has been confirmed among the central and northern regions of Mexico, Central America and the Caribbean.

Finally, analyses combining morphological and genetic characteristics in the continental populations of freshwater copepods (and other zooplankton groups) are still scarce. These kinds of studies are even more rare for insular, freshwater organisms. Our study is the first work in Mexico and the Caribbean region, which combined the genetic and morphological tools in research on the freshwater copepod diversity in insular freshwater bodies.

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**Data Availability Statement:** All sequences the public dataset with the name *Mastigodiaptomus* was created in BOLD database and is available in: DOI: 10.5883/DS-MMASTIGO.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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