Genetic structure and distributional patterns of the genus *Mastigodiaptomus* (Copepoda) in Mexico, with the description of a new species from the Yucatan Peninsula

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Abstract. *Mastigodiaptomus* is the most common diaptomid in the Southern USA, Mexico, Central America and Caribbean freshwaters, nevertheless its distributional patterns and diversity cannot be established because of the presence of cryptic species hidden under wide distributed forms. Herein we study the morphological and molecular variation of the calanoid fauna from two Biosphere Reserves in the Yucatan Peninsula and we describe a new species of the genus *Mastigodiaptomus*. Our findings are compared with other lineages previously found in Mexico. *Mastigodiaptomus siankaanensis* sp.n. is closely related to *M. nesus*, from which can be recognized because of the absence of the spinous process in segment 10 of male A1 and the seta formula and ornamentation of female A1. The mitochondrial cytochrome c subunit I gene (COI) revealed a mean of 0–2.77% K2P divergence within *M. siankaanensis* sp.n. and 14.46–22.4% from other *Mastigodiaptomus* species. Within the new species three different populations were detected, two distributed in close localities (sympatric) and the third consistent with allopatric distribution. The General Mixed Yule Coalescence method (GMYC) delimited eight species of *Mastigodiaptomus* distributed in Mexico. The high diversity and endemism of *Mastigodiaptomus* in the Yucatan Peninsula and Antilles suggest a Neotropical origin of the genus.

Key words. Morphology, Biodiversity, sibling species, sympatric speciation, COI mtDNA, biogeography, Caribbean, Neotropical.

1. Introduction

Copepods are an extraordinary diverse group with respect to their morphologies, physiology, life-strategies and habitat preferences (Boxshall & Defaye 2008; Bron et al. 2011). Among freshwater environments the Diaptomidae Baird, 1850 is the largest and dominant family of the order Calanoida Sars, 1903, including more than 450 species widely distributed in Europe, Asia, America, Africa and, two species in Australia (Boxshall & Jaume 2000; Boxshall & Defaye 2008). Most of the Diaptomidae are planktonic, some are benthic and few species inhabit subterranean waters. The family is also characterized by restricted distribution, where around 90% of the species are endemic to a single biogeographic region (Boxshall & Jaume 2000; Boxshall & Defaye 2008; Barrera-Moreno et al. 2015).

Despite the great diversity of the copepods, their phenotypes tend to be conservative (morphological stasis) (Pesce 1996; Blanco-Bercial et al. 2014) and cryptic speciation seems to be a natural phenomenon, making difficult the separation of species within the group. Numerous efforts have been focused on the development of molecular tools and use of genetic approaches to identify and delimitate copepod species, especially those from marine environments (Lee 2000; Blanco-Bercial et al. 2014). Nevertheless, in continental waters there are few studies dedicated to the evaluation of species boundaries using
Morphological and genetic approaches (Monchenko 2000; Doodson et al. 2003; Alekseev et al. 2006; Elías-Gutiérrez et al. 2008; Thum & Harrison, 2009; Wyngaard et al. 2009; Karanovic & Krajeck 2012; Marrone et al. 2013; Gutiérrez-Aguirre et al. 2014; Barrera-Moreno et al. 2015; Gutiérrez-Aguirre & Cervantes-Martínez 2016) being the traditional morphological work the most used approach in the description and delimitation of species. It is also known that in some cases these called cryptic species are morphologically distinguishable when traditionally overlooked morphological characters are included in the separation of these “pseudo sibling-species” (Marrone et al. 2013); thus the combination of a well defined set of morphological characters, genetic approaches and distributional patterns will lead us to correctly delimitate species within copepods.

In particular, within the Neotropical region the Diaptomidae fauna is highly complex, with many species restricted to small localities such as lakes, reservoirs, wetlands, or particular hydrographic basins. Recent efforts have been made, especially in South America, to clarify not only the taxonomic status of some genera (e.g. Rhacodiaptomus Kiefer, 1936, Argyrodiaptomus Brehm, 1933, Notodiaptomus Kiefer, 1936 and Diaptomus Westwood, 1936) but to establish their geographic distribution (Suárez-Morales et al. 2005; Santos-Silva 2008; Periche-Neves et al. 2013). Mastigodiaptomus Light, 1939 is the most common diaptomid genus in Southern USA, Mexico, Central America and the Caribbean, with limited distributions in the most of the species. Mastigodiaptomus montezumae (Brehm, 1955), M. reidae Suárez-Morales & Elías-Gutiérrez, 2000, M. maya Suárez-Morales & Elías-Gutiérrez, 2000, M. suarezmoralesi Gutiérrez-Aguirre & Cervantes-Martínez, 2013 and M. patzcuarensis (Kiefer, 1938) and the recently described M. cuneatus Gutiérrez-Aguirre & Cervantes-Martínez, 2016 are probably endemic to different aquatic systems from Mexico. Other species with restricted distribution are M. amatitlensis (Wilson M.S., 1941) endemic to Lake Amatitlán in Guatemala, M. purpureus (Marsh, 1907) from Cuba and recorded in Haiti as well (Reid 1996) and M. nesus Bowman, 1986 distributed in Bahamas, Belize and the Yucatan Peninsula. Some other species have wider distributions as M. texensis Wilson M.S., 1953 described in Texas but recorded in north and south of Mexico (Elías-Gutiérrez et al. 2008; Elías-Gutiérrez et al. 2008b) and the widely distributed M. albuquerqueensis (Herrick, 1895) recorded from the Southern USA to Central America, and actually split in two different species (Reid 1997; Suárez-Morales & Elías-Gutiérrez 2000; Suárez-Morales & Reid 2003; Brandonff 2012; Gutiérrez-Aguirre & Cervantes-Martínez 2013; Gutiérrez-Aguirre et al. 2014). The most recent efforts to clarify the taxonomic status of the Mastigodiaptomus fauna used modern integrative approach to delimitate the species (Gutiérrez-Aguirre et al. 2014; Gutiérrez-Aguirre & Cervantes-Martínez 2016). This and previous studies have recognized that the diversity of the genus is underestimated because of the presence of cryptic species coexisting not only in the same biogeographic area, sometimes in the same locality (Elías-Gutiérrez et al. 2008). Therefore, the inclusion of integrative approaches in the study of the Mastigodiaptomus is needed in order to recognize its real diversity and to understand their biogeographic patterns.

Herein, we include morphological and mtDNA COI genetic analyses of the genus Mastigodiaptomus from two Biosphere Reserves from the Yucatan Peninsula in Mexico and the description of a new species of the genus Mastigodiaptomus. Our findings are compared with other lineages previously found in Mexico, in order to clarify their biogeographic associations.

2. Materials and methods

2.1. Study area

Two protected areas of Mexico, both under the category of Biosphere Reserve: Sian Ka’an and Calakmul have been sampled for this study (Fig. 1) (collection permit: PPF/DGOPA-003/15 SEMARNAT-CONAPESCA, Mexico). These reserves represent the most extensive ar-

<table>
<thead>
<tr>
<th>Locality Reserve</th>
<th>Date</th>
<th>Species recorded</th>
<th>Geographic coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aguada Viga Chico</td>
<td>Sian ka’an</td>
<td>20-09-2014</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Savannah 2</td>
<td>Sian ka’an</td>
<td>21-08-2014</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Aguada limite de la reserva</td>
<td>Sian ka’an</td>
<td>23-09-2014</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Arroyo Calamut</td>
<td>Calamut</td>
<td>28-09-2014</td>
<td>M. reidae</td>
</tr>
<tr>
<td>Arroyo Aguada Grande</td>
<td>Calamut</td>
<td>28-09-2014</td>
<td>M. reidae</td>
</tr>
<tr>
<td>Pokora</td>
<td>Sian ka’an</td>
<td>29-08-2015</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Dolum</td>
<td>Sian ka’an</td>
<td>29-08-2015</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Aguada limite de la reserva</td>
<td>Sian ka’an</td>
<td>03-10-2015</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Aguada Viga Chico</td>
<td>Sian ka’an</td>
<td>04-10-2015</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Savannah Playon</td>
<td>Sian ka’an</td>
<td>06-10-2015</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Kohunlich</td>
<td>—</td>
<td>31-07-2005</td>
<td>M. siankaanensis sp.n.</td>
</tr>
<tr>
<td>Kohunlich</td>
<td>—</td>
<td>31-07-2005</td>
<td>M. reidae</td>
</tr>
<tr>
<td>Tres Garantias</td>
<td>—</td>
<td>22-05-2011</td>
<td>M. siankaanensis sp.n.</td>
</tr>
</tbody>
</table>

Table 1. Sampling localities in Calakmul and Sian ka’an Biosphere Reserves and additional records of M. siankaanensis sp.n. (*) indicates the type locality.
estouP

with 96% ethanol at 4°C.

Mastigodiaptomus

and females of the genus Mastigodiaptomus were sorted from samples and preserved in vials overnight, according to protocol (Michels & Bontzow 2010). For ventral and dorsal habitus views, the undissected animals were prepared on slides using Karo® light corn syrup as mounting medium so that the animals were intact and not compressed during the scanning process. Dissected appendages were mounted on individual slides with glycerine. The material was examined using a Leica TCS SP5 equipped with a Leica DM5000 B upright microscope and 3 visible-light lasers (DPSS 10 mW 561 nm; HeNe 10 mW 633 nm; Ar 100 mW 458, 476, 488 nm; HeNe 10 mW 633 nm; Ar 100 mW 458, 476, 488 nm; and 514 nm), combined with the software LAS AF 2.2.1. (Leica Application Suite Advanced Fluorescence).

Images were obtained using 561 nm excitation wave length with 80% acousto-optic tunable filter (AOTF). Series of stacks were obtained, collecting overlapping optical sections throughout the whole preparation with optimal number of sections according to the software. The acquisition resolution was 2048 × 2048. Final images were obtained by maximum projection, and CLSM illustrations were composed and adjusted for contrast and brightness using the software Adobe Photoshop CS4.

Abbrivations used: A1 = antennule, A2 = antenna, Ae = aesthete, Cph = cephalothorax, cnp = endopod, exp = exopod; Md = mandible, Mx1 = maxillula, Mx2 = maxilla, Mxp = maxilliped, Ms = modified seta, P1-P5 = legs 1 to 5, Urs = urosomite(s), vs = vestigial seta. Caudal setae labeled as follows: II – anterolateral (innermost) caudal seta; III – posterolateral (outermost) caudal seta; IV – outer terminal (terminal median external) caudal seta; V – inner terminal (terminal median internal) caudal seta; VI – terminal accessory (innermost) caudal seta; VII – dorsal seta; nomenclature follows Huys & Boxshall (1991). The terms furca and telson are used following Schminke (1976) and setae formula modified from PerBiche-neves et al. (2013).

2.3. COI sequencing and genetic analysis

DNA extractions from 33 specimens were carried out using 40 µl Chelex (InstaGene Matrix, Bio-Rad) according to the protocol (Estoup et al. 1996) and directly used as DNA template for PCR. A 658 base-pair region of mtDNA COI was amplified using the primers LCO-
1490 (Folmer et al. 1994): GGTCAACAAATCATATAAAGATATTGG and Cop-COI-2198R (Bucklin et al. 2010): GGGTGACCAAATATACARAA. The PCR protocol was 94°C for 5 min, 94°C for 45 s, 45°C for 45 s, and 72°C for 50 s, during 38 cycles and as final elongation 72°C for 3 min. PCR was carried using PhReTaQ Ready-To-Go PCR Beads (GEHealthcare) in 25 µl volume containing 22 µl nuclease-free water, 0.5 µl of each primer (10 pmol/µl) and 2 µl DNA templates. All PCR products were checked by electrophoresis on a 1% agarose/TAE gel containing 1% GelRed. PCR products were purified using ExoSap-IT PCR Product Cleanup (Affymetrix, Inc) at 37°C followed by an incubation period of 80°C and sequencing were carried out by Macrogen (Amsterdam, Netherlands). Forward and reverse sequences for each specimen were assembled, edited and checked for correct amino acid translation frame using Geneious 9.1.7 (created by Biomatters; available from http://www.geneious.com). All sequences were searched against GenBank nucleotide database using BLASTN (Altschul et al. 1990). Sequences of closely related species were downloaded from NCBI and included in the analyses comprising M. siankaanensis sp.n. (4 specimens); M. montezumae (36 specimens), M. reidae (17 specimens), M. albuquerqueenis (21 specimens), M. nesus (4 specimens), M. texensis (3 specimens), M. patzuarensis (22 specimens) and M. cuneatus (1 specimen).

Supplementary 1 lists the GenBank accession numbers downloaded from NCBI. Genbank accession numbers of the sequences obtained during this study are as follows:

**M. siankaanensis sp.n.**:
MK080113, MK080114, MK080115, MK080116, MK080117, MK080118, MK080119, MK080120, MK080121, MK080122, MK080123, MK080124, MK080125, MK080126, MK080127, MK080128, MK080129, MK080130, MK080131, MK080132, MK080133, MK080134, MK080135, MK080136, MK080137, MK080138, MK080139;

**M. reidae**:
MK080140, MK080141, MK080142, MK080143, MK080144, MK080145.

All DNA sequences including sequences available from this study and downloaded from GenBank were aligned using MAFFT v7.017 under G-INS-i algorithm (Katoh & Toh 1990) and alignment were further edited manually. A Bayesian analysis employing the K2P substitution model were conducted using MrBayes MPI version (Ronquist & Huelsenbeck 2003; Altekar et al. 2004). Posterior probabilities were estimated using 5,000,000 generations with sampling frequency of every 1000 trees through four simultaneous Markov Chains Monte Carlo. The consensus tree with median branch lengths was made, discarding the 1250 first trees. MEGA 7 (Kumar et al. 2015) has been used in order to calculate the K2P genetic variations of mtDNA COI within and between species. The General Mixed Yule Coalescent model (GMYC) (Pons et al. 2006; Monaghan et al. 2009) has been used as species delimitation method in which the simple threshold approach assumes that there is a threshold time, before which all nodes reflect diversification events (inter-specific) and after which all nodes reflect coalescent events (intra-specific). The number of species obtained by this approach is thus estimated by this threshold time. The GMYC method implemented in the “splits” package for R was applied to the COI ultrametric tree obtained with BEAST v1.8.3 (Drummond et al. 2016). Nucleotide diversity (r) and neutrality test using Tajima’s D (Tajima 1989) were calculated with PopART (Bandelt et al. 1999). AMOVA was performed to calculate genetic variations between geographically separated groups. Statistical parsimony method was used to construct a Minimum Spanning haplotype network with PopART (http://popart.otago.ac.nz).

### 3. Results

Two species of Calanoida were detected from samples obtained during this survey: M. reidae and a new species for science, M. siankaanensis sp.n. Description is based on morphology, mtDNA COI genetic variations and distributional patterns. Additionally, we include an overview of the Mexican records of the genus, their distributional patterns and genetic divergence.

#### 3.1. Molecular diversity and population structure

A total number of 33 specimens have been sequenced in this study comprising 27 sequences of mtDNA COI from M. siankaanensis sp.n. from Sian ka’an Biosphere Reserve and six sequences of mtDNA COI of M. reidae from Calakmul Biosphere Reserve. The alignment is provided for the 33 COI sequences of this study together with the 85 sequences of all the GenBank COI records available from this genus (supplementary 1). Maximum and minimum length of resulting alignment was 670 and 592 bp, respectively. Table 2 shows minimum and maximum inter- and intra-specific genetic divergence calculated by K2P substitution model for distinct clades of *Mastigodiaptomus* including GenBank available COI sequences of this genus. Kimura-two-parameter distance model revealed the minimum and maximum of 0 – 2.77% diversity within *M. siankaanensis* sp.n. and 14.46 – 22.4% from other *Mastigodiaptomus* species. *Mastigodiaptomus reidae* showed 0 – 2.51% and 17 – 24.18% minimum and maximum K2P distance within species and compare to other species correspondingly. Figure 2 indicates the phylogram generated by Bayesian analyses of COI sequences from *Mastigodiaptomus* species including GenBank sequences in which the branches are collapsed into the clade level (supplementary 2 shows the complete COI tree). The number of eight species (Fig. 2) has been identified with GMYC.
### Table 2. Percentage of maximum and minimum K2P inter- and intra-specific genetic divergence shown for defined clades of *Mastigodiaptomus* species.

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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. siankaanensis</em> sp.n.</td>
<td>2.05 – 2.77</td>
<td>0 – 1.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. reidae</em></td>
<td>19.26 – 19.89</td>
<td>17.96 – 19.26</td>
<td>17.17 – 19.03</td>
<td>0 – 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. texensis</em></td>
<td>2.77 – 3.49</td>
<td>2.77 – 3.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. montezumae</em> (Clade 1)</td>
<td>17.1 – 19.98</td>
<td>17.98 – 18.32</td>
<td>23.20 – 25.64</td>
<td>21.59 – 21.73</td>
<td></td>
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</tbody>
</table>

**Note:** Figs. 4–14: Mastigodiaptomus Light, 1939

3.2. Description

*Mastigodiaptomus siankaanensis* sp.n.

**Order:** Calanoida

**Family:** Diaptomidae

**Species:** *Mastigodiaptomus siankaanensis* Light, 1939

**Genus:** *Mastigodiaptomus*

**Species:** *Mastigodiaptomus siankaanensis* sp.n.

**Clade 1:** September 23, 2014, by Nancy F. Mercado-Salas.

**Clade 2:** Aguada limite de la reserva, Sian ka’an Biosphere Reserve, Quintana Roo, Mexico (19°42′32.6″N 87°49′40.1″W)

**Paratypes:** ECO-CH-Z-10101), same site, date and collector.

**Additional material:** See Table 1.
**Type locality.** Aguada Limite de la Reserva, Station 1, Sian ka’an Biosphere Reserve, Quintana Roo, Mexico (19°42′32.6″N 87°49′40.1″W).

**Etymology.** The name is after the Sian ka’an Biosphere Reserve in which the specimens were collected; Sian Ka’an in Mayan language means “heaven’s door” or “place where heaven begins”.

**Description.** Female: Total body length 1571 µm (x = 1596.84, n = 13) excluding furca, fifth pediger without dorsal process; Urs 22% of body length. Body symmetrical in dorsal view, prosome slightly wider at distal third (Figs. 4A, 10A). In lateral view (Fig. 4B) the body is arched downwards. Rostrum with long rostral points. Thoracic wings asymmetrical, left longer than right, both bearing 2 strong spinules. Urs 3-segmented;
genital-double somite bearing 1 large spine on right margin, left margin with a slightly smaller spine (Figs. 5A, 12E). Genital urosome bearing a rounded protuberance on ventral surface (Fig. 5B). Telson about 3.0 × longer than preanal somite and with anal operculum rounded. Furca 2 × longer than wide, bearing hairs on outer and inner margins, caudal setae subequal in length and biserially plumose (Fig. 5A).

A1 (Figs. 5C, 11A,B): 25-segmented; tip of last segment exceeding total length of furca. Armament per segment: 1(1ms), 2(3ms + 1ae), 3(1ms), 4(1ms), 5(1ms + 1ae), 6(1ms), 7(1ms + 1ae), 8(1ms + 1sp), 9(2ms + 1ae), 10(1ms), 11(2ms), 12(1ms + 1sp + 1ae), 13(1ms), 14(1ms + 1ae), 15(1ms), 16(1s + 1ae), 17(1ms), 18(1s), 19(1ms + 1ae), 20(1ms), 21(1s), 22(2s), 23(1s), 24(2s), 25(5s + 1ae). Presence of hairs on inner margin of segments 2–15. A1 armament provided represent maximum setation found in holotype and paratypes, Fig. 5C illustrates holotype.

Table 3. Population analyses based on statistical parsimony method, provided for the COI sequences of four different species of *Mastigodiaptomus*. **: Highly significant; *: Significant.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of Polymorphic Sites</th>
<th>Nucleotide Diversity</th>
<th>No. of Haplotypes</th>
<th>Fixation Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. siankaanensis</em> sp.n.</td>
<td>19</td>
<td>0.0032</td>
<td>10</td>
<td>0.801**</td>
</tr>
<tr>
<td><em>M. reidae</em></td>
<td>20</td>
<td>0.0122</td>
<td>8</td>
<td>0.204**</td>
</tr>
<tr>
<td><em>M. montezumae</em></td>
<td>26</td>
<td>0.014</td>
<td>12</td>
<td>0.042*</td>
</tr>
<tr>
<td><em>M. albuquerquensis</em></td>
<td>38</td>
<td>0.0073</td>
<td>13</td>
<td>0.846**</td>
</tr>
</tbody>
</table>

Fig. 4. *Mastigodiaptomus siankaanensis* sp.n. female, holotype. A: habitus, dorsal view. B: habitus, lateral view. Scale bar = 250 μm.

→ Fig. 5. *Mastigodiaptomus siankaanensis* sp.n. female, holotype. A: Urs, telson, furca, dorsal view. B: Urs, telson, lateral view. C: A1-right. Scale bars, A,B = 125 μm; C = 100 μm.
A2 (Figs. 6A, B, 11C): Coxa with 1 strong long seta. Basis slightly elongated with 2 subequal setae on inner margin. 2-segmented enp, enp-1 bearing 2 subequal setae on inner margin and 1 row of strong spinules on outer margin; enp-2 with 2 lobes, outer lobe with tiny spinules on outer margin and bearing 7 setae, inner lobe with 9 setae. Exp 7-segmented, setation pattern: 1, 3, 1, 1, 1, 1, 4. Second segment with 2 pseudosegments (arrow in Fig. 6B).

Md (Figs. 6C, 11D): Gnathobase with 7 rounded teeth, innermost margin with 1 spinulose seta, outermost margin with 1 rounded lateral projection. Coxa bare, basis with 4 setae. Enp 2-segmented; enp-1 bearing 4 setae, enp-2 with row of small spinules on outer margin and with 9 setae on apical margin. 4-segmented exp; setation pattern: 1, 1, 1, 3.

Mx1 (Figs. 6D, 11E): Precoxal arhrite with 15 spiniform setae, coxal epipodite bearing 9 long setae; coxal endite quadrangular with 4 apical setae. Basis with 1 basal endite bearing 4 setae, internal lobe bearing 4 setae and basal exite represented by 1 seta. 2-segmented enp, enp-1 and enp-2 armed with 4 setae each. Exp 1-segmented, bearing 6 setae.

Mx2 (Figs. 6E, 11E): Praecoxa with 2 lobes, first lobe with 5 setae and bearing small basal spinules; second lobe bearing 3 setae and small basal spinules. Coxa with 2 endites both with basal spinules; first endite bearing 2 long setae and second with 3 long setae. Basis with well develop allobasis and 1 distal lobe. Allobasis bearing 4 long setae; distal lobe with 1 long seta. Enp 2-segmented; enp-1 with 1 long seta and enp-2 bearing 4 setae.

Mxp (Figs. 6F, 12A): Praecoxa and coxa fused in 1 long segment. Praecoaxal endite bearing 1 seta; coxa with 3 coxal endites, first bearing 2 setae and small basal spinules, second lobe with 3 setae, and third with 4 setae. Coxal distal margin ornamented with small slender spinules. Basis proximal inner margin ornamented with row of small slender spinules and 3 setae on distal margin, distalmost seta longer than others. 6-segmented enp, setation pattern: 2, 3, 2, 1, 1+1, 4.

P1 (Figs. 7A): Coxa with plumose seta on inner margin, reaching proximal end of enp-1. Basis with group of long slender spinules on outer margin (arrow in Fig. 7A). Enp 2-segmented, enp-2 about 1.6 × longer than enp-1. Enp-1 bearing 1 inner seta, exp-2 with 3 inner, 2 apical and 1 outer setae, all plumose. Exp 3-segmented, segments progressively tapering: exp-1 with 1 inner seta and 1 small outer spine, exp-2 with 1 long inner seta and; exp-3 with 2 long inner setae, 3 apical setae (outermost with tiny spinules on outer margin and plumose on inner margin) and, 1 small outer spine.

P2 (Fig. 7B): Outer margin of basis, coxa and enp-1 ornamented with groups of tiny spinules (arrow in Fig. 7B). Coxa with plumose seta on inner margin, exceeding proximal end of enp-1. Enp 3-segmented, segments progressively tapering; exp-1 bearing 1 inner seta, enp-2 with 2 inner setae and, enp-3 with 3 inner, 2 apical and 2 outer setae, all homogeneously plumose, row of small spinules near insertion of 2 apical setae. Exp 3-segmented, exp-1 and exp-3 about the same length, exp-2 slightly shorter (about 0.77 × as long as the other segments). Exp-1 with small spinules on outer margin, with 1 outer spine and 1 inner seta; exp-2 bearing 1 outer spine and 1 long inner seta; exp-3 bearing 1 outer spine, 3 apical long setae (outermost seta with spinules on outer margin and plumose on inner margin) and, 3 long inner setae.

P3 (Fig. 7C): Coxa with plumose seta on inner margin, reaching proximal end of enp-1. Exp 3-segmented, segments progressively tapering; exp-1 bearing 1 inner seta, exp-2 with 2 inner setae and, exp-3 with 2 long inner, 3 long apical and 2 short outer setae, all homogeneously plumose. Exp 3-segmented, exp-1 and exp-3 about the same length, exp-2 slightly shorter (about 0.72 × as long as the other segments). Exp-1 with 1 outer spine and 1 inner seta, exp-2 bearing 1 outer spine and 1 long inner seta, exp-3 bearing 1 outer spine, 3 apical long setae (outermost seta with spinules on outer margin and plumose on inner margin) and, 3 long inner setae.

P4 (Figs. 7D, 12B): Coxa with plumose seta on inner margin, not reaching proximal end of enp-1, basis with small outer seta (arrowed in Fig. 7D). Exp 3-segmented, segments progressively tapering; exp-1 bearing 1 inner seta, exp-2 with 2 long inner setae and, exp-3 with 2 inner, 3 apical and 2 short outer setae, all setae homogeneously plumose. Exp 3-segmented, all about the same size.

P5 (Figs. 7E, 12C,D): Coxa symmetrical, bearing a conical seta on outer margin; basis triangular. 3-segmented exp: exp-1 elongate; exp-2 armed with a strong claw, ornamented in both margins with strong spinules, outer margin with 1 small strong spine on distal margin (arrow in Fig. 7E); and exp-3 bearing 1 long strong seta and 1 short spine. Exp 2-segmented, total length of exp shorter than exp1; exp2 with 2 long setae plus row of hair-like setae on distal margin, exp1 nude.

Male: Total body length 1375 µm (x = 1387.25, n = 8) excluding furca. Body slender, eph wider at prosomal region in dorsal view (Figs. 13A,B). Complete suture between pedigerous somites 4–5. Left thoracic wing not projected, bearing 1 small spine; right thoracic wing slightly projected, with 1 ventral spine and 1 thin dorsal spine. Right margin of Urs-1 with 1 spine.

A1-right (Figs. 8A, 14B,C): 22 expressed segments, armament per segment: 1(1ms), 2(3ms +1ae), 3(1ms), 4(1ms), 5(1ms), 6(1ms), 7(1ms), 8(1ms +1sp), 9(2ms), 10(1ms +1s), 11(1ms +1sp), 12(1ms +1ae), 13(1s +1sp), 14(1s +1ms +1sp), 15(1s +1ms +1ae +1sp), 16(2s +1ae +1sp), 17(1s), 18(0), 19(1ms +1sp), 20(3s +1sp), 21(1s), 22(6s).

Spinous processes on segments 11, 13, 14, 15 and 16 (arrow in Figs. 8B, 14C). Segment 20 with an acute long process distally, reaching proximal margin of segment 21 (arrow in Figs. 8B, 13C). Segments 17 and 18 with hyaline process on dorsal margin (arrow in Fig. 14C).

A1-left (Figs. 8B, 14A): 25 expressed segments, armament per segment: 1(3s +1ae), 2(2ms +1ae), 3(1ms +1ae), 4(1ms), 5(1ms +1ae), 6(1ms), 7(1ms +1ae), 8(1ms +1s), 9(1ms +1ae), 10(1s), 11(2ms), 12(1ms +1sp +1ae), 13(1ms), 14(1ms +1ae), 15(1ms), 16(1s +1ae), 17(1s), 18(1s), 19(1ms +1ae), 20(1ms), 21(1s), 22(2s), 23(3s), 24(1s), 25(3s +1ae).

Mouthparts and P1–P4 as in females.

P5-right (Figs. 9E, G, 14D,E): Caudal side. Long distal seta on coxa (arrow in Fig. 9E). Exp longer than exp-1, exp represented by a single segment (Fig. 9G), bearing a row of slender spines on posterior edge. Exp-2 1.6 × longer than wide with one slightly curved hyaline membrane; lateral spine slightly curved, as long as total length of exp-2, bearing small spinules on inner margin. Terminal claw strong, curved and bearing small spinules on inner margin, about 2.8 × longer than exp-2.

P5-left (Figs. 9E,F, 14D,E): Reaching posterior margin of right exp-1. Long distal seta on coxa. Basis slightly elongated, with subterminal lateral seta, reaching distal end of segment. Exp unsegmented, exceeding medial margin of exp-1, and bearing a row of slender spines on posterior edge. Exp-1 longer than wide, 1.7 × longer than exp-2; exp-2 with 1 pad-like process ornamented with long slender setules (arrow in Fig. 9F), exp-2 tapering distally and with 3–4 short chitinous denticles.

Remarks. Mastigodiaptomus siankaanensis sp.n. is established within the genus Mastigodiaptomus because of the presence of the following characters: (1) A1 segment 11 of females and males bearing 2 setae; (2) coxa of female P5 with long, spatulated seta; (3) 1 outer spine on exp-1 P1 in both females and males; (4) male with P5 right exp longer than exp-1; and (5) male A1 with spiniform process on segments 10, 11, 13–16. Dorsal
Fig. 7. *Mastigodiaptomus siankaanensis* sp.n. female, holotype. A: P1-right. B: P2-left. C: P3-right. D: P4-right. E: P5. Scale bars = 100 µm.
process on the last thoracic segment absent in all specimens analyzed, this character was recognized as a typical morphological character for the genus (Suárez-Morales et al. 1996). Nevertheless recent studies have shown that its presence is variable in most of the species of Mastigodiaptomus with the exception of M. purpureus (where is always present), then it should not been used as a key character in the definition of the taxon (Gutiérrez-Aguirre & Cervantes-Martínez 2013).

The distinguishing characters of *M. siankaanensis* sp.n. include the presence of modified and vestigial setae on female A1; inner margin of segments 2, 4–15 in female A1 ornamented with fine hairs; P2 with coxa, basis and enp-1 ornamented with fine spinules; the location of aesthetascs in both right and left males A1; the absence of a spinular process on segment 10 of male right A1 (represented by a small but strong spine) and; the absence of hyaline process on male P5 basis. A detailed morphological comparison among *Mastigodiaptomus* known species is given in Table 4.

Fig. 8. *Mastigodiaptomus siankaanensis* sp.n. male, allotype. A: A1-right. B: A1-left. Scale bars = 100 µm.

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<td>Total body length (µm)</td>
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<td>1500 – 1700</td>
<td>1470 – 1600</td>
<td>2300 – 2470</td>
<td>1375 – 1825</td>
<td>1360 – 1720</td>
<td>1440 – 1540</td>
<td>925 – 1159</td>
<td>1500 – 1600</td>
<td>ND</td>
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<td>Dorsal hump</td>
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<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
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<tr>
<td>L/W genital-double somite</td>
<td>1.08 – 1.1</td>
<td>1.82</td>
<td>2.0</td>
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<td>1.23</td>
<td>1.23</td>
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<td>Cph/Urs ornamentation</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
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<td>Present</td>
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<td>Present</td>
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<tr>
<td>L/W Furca</td>
<td>2.0</td>
<td>1.6</td>
<td>1.0</td>
<td>2.3</td>
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<td>1.3</td>
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4. Discussion

The Neotropical genus *Mastigodiaptomus* is characterized by its distinct morphological features, particularly the presence of a dorsal hump and a ventral hump. The genus is known for its diversity, with 12 described species distributed throughout the Neotropical region, including Mexico. The new species described here, *Mastigodiaptomus siankaanensis* sp.n., is the first to be found in the Yucatan Peninsula. The species is characterized by a unique combination of morphological traits, including the presence of a dorsal hump and a ventral hump, which are not present in other species of *Mastigodiaptomus*. The presence of these traits in the new species indicates that it is a distinct and unique species, deserving of specific recognition. The species is likely to be found in freshwater habitats, as is typical for *Mastigodiaptomus* species. Further studies are needed to determine the distribution and ecology of this new species in the Yucatan Peninsula. The discovery of this new species adds to the understanding of the diversity of *Mastigodiaptomus* species in the Neotropical region, and highlights the need for continued research to explore the diversity of these copepods in Mexico.
populations from Quintana Roo, Arroyo Calakmul and Arroyo Aguada Grande with low inter population $F_{st}$ index of 0.204 which confirms their sympatric distribution.

The third haplotype of *M. siankaaensis* sp.n. is distributed in a geologically younger area of the Peninsula where many stages of transgressions and regressions took place during Upper Oligocene, Middle and Upper Miocene and Lower Pliocene. COI mtDNA genetic divergence between three different populations of *M. siankaaensis* sp.n. investigated in this study (2.5 – 2.77% with high $F_{st}$ index of 0.801) showed the same pattern as inter-population average distance of lacustrine copepods from Mexico (BARRERA-MORENO et al. 2015). Morphologically, the specimens of *M. siankaaensis* sp.n. from the south of Quintana Roo (Tres Garantias and Kohunlich) were similar to those from Sian ka’an Biosphere Reserve, with the exception of the lack of P2 ornamentation. The major difference found among the phenotypes was the size. Southern populations are smaller, with and average total length of 1283 ± 6 µm (n = 3) for females and 1050 ± 27 µm.

**Fig. 9.** *Mastigodiaptomus siankaaensis* sp.n. male, allotype. **A:** P1-right. **B:** P2-right. **C:** P3-right. **D:** P4-left. **E:** P5-right. **F:** P5-left. **G:** P5-right enp. Scale bars, A – F = 100 µm, G = 25 µm.
(n = 4) for males in Tres Garantías. In Kohunlich they are intermediate in size (1545 and 1260 µm for females and males). These differences can be possibly explained on basis of predation and competence. Tres Garantías is a tropical lake where plenty of fish and invertebrate predators as rotifers, insect larvae and corixids live, whereas Kohunlich pond lacks major predators as fishes, its uniqueness could be associated to the coexistence with two species of the genus: *M. reidae* and another possibly undescribed species which we did not manage to collect for this study. In the latter case, *M. siankaanensis* sp.n. is the smallest one among the three. In congruence with the study of Barrera-Moreno et al. (2015) about copepod population from neighbor lakes in Mexico, there is no shared COI haplotype from different population of *M. siankaanensis* sp.n. from this study, which further indicates the absence of gene flow and restricted dispersion in this area. The new species is closely related to *M. nesus*, *M. texensis* and *M. reidae* which are distributed in the Yucatan Peninsula. *Mastigodiaptomus nesus* was recorded in several localities near to the distributional area of *M. siankaanensis* sp.n. in the Sian ka’an Biosphere Reserve (especially in “cenotes” or sinkholes) and in the northwestern area of Yucatan State (Suárez-Moraless et al. 1996). It is important to highlight that the new species was only found in smaller water bodies such as temporal and permanent wetlands and aguadas; it was never found in the cenotes surveyed during this work, this could represent an ecological adaptation very specific for each of the two species. *Mastigodiaptomus siankaanensis* sp.n. can be morphologically distinguished from *M. nesus*, *M. texensis* and *M. reidae* because of the absence of the spinous process in segment 10 of male A1, in the new species this process is represented by a strong but small spine while in the other species the process is strongly developed. Another character that differentiates species is the absence of the hyaline process on male P5 basis in the new species and, a clearly sub-quadrangular process in *M. nesus*. Females of *M. siankaanensis* sp.n. also are easily separated from those of *M. nesus*, *M. texensis* and *M. reidae* by the possession of the A1 strongly ornamented on inner margin in segments 2, 4 – 15. The presence of aesthetascs, modified setae and vestigial setae in both females and males should be included in posterior studies since they could represent useful characters in the differentiation of species within the genus.

Fig. 10. *Mastigodiaptomus siankaanensis* sp.n. female, paratype (CLSM). A: habitus, dorsal view. B: habitus, ventral view. Scale bar = 200 µm.
The origin of the genus *Mastigodiaptomus* has been suggested in the upper Neotropical region as result of the diversification of Neartic diaptomids which dispersed and radiated in and after the Proto-Antilles (70 Mya) (Suárez-Morales & Reid 2003; Suárez-Morales 2003). This process could originate species with restricted distributions in the Caribbean, the Antilles and the Yucatan Peninsula as *M. reidae*, *M. maya*, *M. nesus*, *M. purpureus* and the herein described *M. siankaanensis* sp.n. The dry weather periods during the late Pleistocene and throughout the Holocene could have been an additional factor to limit the recent dispersal of the tropical *Mastigodiaptomus* and could cause the isolation especially of the third haplotype of *M. siankaanensis* sp.n. favoring its disjunctive distributional pattern. Records of *M. albuquerqueensis* and *M. texensis* in the eastern coast of the Yucatan Peninsula (Tulum-Playa del Carmen) should be re-analyzed with the new tools used in this work, in order to clarify their taxonomical identity.

The recently described *M. suarezmoralesi* from Chiapas (Usumacinta Province-Neotropical region) and *M. amaitilaensis* could represent a different radiation process that has been associated with the development of rivers and terraces in the Usumacinta basin during Pleistocene (Gutiérrez-Aguirre & Cervantes-Martínez 2013; Gutiérrez-Aguirre & Suárez-Morales 2001). Their distributional patterns and phylogenetic associations should be addressed by future studies that include new collecting campaigns in Chiapas and Guatemala. The presence of *M. albuquerqueensis* (as a complex) and recently redefined *M. patzcuarensis* in Mexico from north to south supports the hypothesis of an intense radiation of the genus from the Antilles and the Yucatan Peninsula (Suárez-Morales & Reid 1998). Unfortunately,
copepods hardly fossilize giving really few direct records of them, which obscures the historical biogeography and lineage divergence times for this group (Holyńska et al. 2016), allowing only to conclude mostly based on the actual distribution of the species.

Presumed sister species which are morphologically similar and allopatrically or parapatrically distributed have lead several taxonomist and biogeographers to suggest the Pleistocene as an important time for diaptomid radiation (Gutiérrez-Aguirre & Cervantes-Martín 2013; Gutiérrez-Aguirre & Suárez-Morales 2001; Stemberger 1995). However more recent studies have tested the hypotheses of Pleistocene divergence and speciation within the genus Skistodiaptomus Light, 1939 using mitochondrial (COI, cytochrome b, 16S) and nuclear (ITS) phylogenies and comparing them with molecular clock calibrations available for other crustaceans (Thum & Harrison 2009). Among the main results, those authors found that DNA sequence divergences among the different species analyzed do not support the hypothesis of Pleistocene speciation inferred from the current parapatric distributions and morphological similarities. Skistodiaptomus sequence divergences suggested that speciation within the genus started much earlier, during the Miocene (10 – 20 Mya) and that Pleistocene play an important role in the divergence within different clades (haplotypes) of some species as S. pallidus (Herrick, 1879). This hypothesis seems to be suitable for some species of the genus Mastigodiaptomus (M. maya, M. reidae and M. siankaanensis sp.n.) which are distributed in the southern part of the Peninsula (emerged even earlier, since the Paleocene). Probably the third haplotype of M. siankaanensis sp.n. evolved later (after Miocene or during Pleistocene) as the inner clades of Skistodiaptomus pallidus suggested by.

Fig. 12. Mastigodiaptomus siankaanensis sp.n. female, paratype (CLSM). A: Mxp. B: P4-left. C: P5, caudal view. D: P5, frontal view. E: genital-double somite, ventral view. Scale bars, A,C – D = 50 µm; B = 100 µm; E = 200 µm.
THUM & HARRISON (2009). Genomic investigation in this genus and dating based on copepod material could test the validity of the above mentioned hypothetical distribution patterns, that in the present work are based only on the geographic distribution of the species.

Until recent years, *M. albuquerquensis* was considered the most widely distributed species of the genus ranging from the south of the United States to the Yucatan Peninsula in Mexico nevertheless deeper morphological examination combined with genetic approaches have shown that in fact it is a complex of species probably with restricted distributions (GUTIÉRREZ-AGUIRRE et al. 2014). Our results from GMYC delimitation method have confirmed a single species with three derived clades within this species together with *M. patzcuarensis* as a close separate species (Fig. 2), in congruent with previous study (GUTIÉRREZ-AGUIRRE et al. 2014) with remarkably high between-species K2P COI divergence (5.21–9.68%). Clade 3 from *M. albuquerquensis* distributed in North of Mexico (Chihuahua and Durango States) and two additional lineages (clades 1 and 2), one distributed in Durango State (northwest side) and the other in Zacatecas State (central east side, more to the south). In the most recent publication about *Mastigodiaptomus* fauna, it was stated that specimens from Zacatecas (clade 2) exhibited morphological differences with respect to clade 3 assigned as *M. albuquerquensis s.str.* by GUTIÉRREZ-AGUIRRE et al. (2014). According to the GMYC result of this study, slightly high mean K2P distances between these two different clades (3.26–4.78%) can be interpreted as intra-species variation. Our result further showed their restricted distribution pattern, as different lineages of *M. albuquerquensis* are distributed in semi-desertic and desertic areas in the North of Mexico.
and two of them shown sympatric distributions (Elías-Gutiérrez et al. 2008) which can be an indication of high inter population variabilities ($F_{st}=0.846$; Table 3).

It was suggested that the closely related species *M. patzcuarensis* which includes records from Guanajuato, Mexico State, Michoacan and Puebla, represents two sibling species, one named as *M. patzcuarensis* and the other as *M. cf. albuquerquensis* (Gutiérrez-Aguirre et al. 2014); however our result rejected this assumption according to the GMYC delimitation following relatively low COI genetic distances between above mentioned lineages of *M. patzcuarensis* (0–1.08%; not shown here). The COI mtDNA analyses retrieve another *Mastigodiaptomus* species from North Mexico, *M. cuneatus* (Gutiérrez-Aguirre & Cervantes-Martínez 2016). The general distribution of *M. albuquerquensis*, *M. patzcuarensis* and *M. cuneatus* further supports allopatric distributions, the *M. albuquerquensis* and *M. cuneatus* including all records from north of Mexico (semi-desertic and desertic areas) and the *M. patzcuarensis* associated to the Mexican Plateau (including temperate areas).

Another species apparently restricted to the central and north of Mexico is *M. montezumae*, described from Hidalgo State and later recorded in Mexico, Aguascalientes, Guanajuato, Durango and Sinaloa states. This species has been mostly co-existed with other diaptomids as *M. albuquerquensis*, *Leptodiaptomus novamexicanus* (Herrick, 1895) and *L. siciloides* (Lilljeborg in Guerne & Richard, 1889) (Dos Santos-Silva et al. 1996). The mtDNA COI analyses revealed two clades within *M. montezumae* differed by the range of 2.77–3.49% genetic differences with sympatric distributions, coexisting even in the same locality in the central highland plateau (above 1500 m a.s.l.) (Mexico State). The mtDNA COI analyses revealed two clades within *M. montezumae* differed by the range of 2.77–3.49% genetic differences with sympatric distributions, coexisting even in the same locality in the central highland plateau (above 1500 m a.s.l.) (Mexico State). Morphological differences between these two clades have not been reported so far and our study further support a single species according to GMYC (Fig. 2). Population structure of this species cannot be discussed here due to only a single haplotype
was available from Aguascalientes State in GenBank (Fig. 3). *Mastigodiaptomus montezumae* is clearly differentiated from its congeners by distinct short projection on segment 20 of male A1, which is long and acute in most of the species and wide knob-like in *M. maya*, and the mammoniform projection ending in a blunt spine on P5 right basipod. The recently described *M. cuneatus* morphologically seems to be related with *M. amatitlaensis*, nevertheless these species are distributed in completely different geographic areas, the first in Northern Mexico and the second in Guatemala (*Gutierrez-Aguirre & Cervantes-Martinez* 2016). Genetically, *M. cuneatus* is closest to *M. albuquerqueensis* and *M. patzcuarensis*, however genetic associations are hard to stabilise because of the lack of sequences of *M. cuneatus* (only one sequence available), and should be addressed in posterior works.

The distribution of microcrustaceans in freshwater ecosystems was explained, until recent years, by the “Cosmopolitan Paradigm”, where scarce morphological differentiation among conspecific populations and the high capacities for passive dispersal allowed them to colonize new areas, keeping an extensive gene flow across their distributional ranges (*Marrone et al. 2013*). However, this idea has been challenged by the “Monopolization Hypothesis”, where founder effect, rapid local adaptation and resilience against newcomers are combined, restricting the gene flow and restricting the species to smaller areas with specific adaptations (*De Meester et al. 2002*). The “Monopolization Hypothesis” provided the theoretical basis to explain the distribution and high genetic differences in presumed conspecific freshwater diatomoids of the genus *Ociddiaptomus* Borutsky in Borutsky, Stepanova & Kos, 1991 (*Marrone et al. 2013*) and fits to the distribution of the *Mastigodiaptomus* species, where the species radiation seems to be more related to local adaptations than to extreme dispersal capacities.

*Mastigodiaptomus* is a genus that probably originated in the Neotropics (upper Neotropics) and dispersed and radiated in and after the Proto-Antilles to the Yucatan Peninsula. This hypothesis and the Miocene or Pleistocene radiation of the genus should be further investigated for the origin and the phylogenetic relationships of the different species within the genus.

### 5. Acknowledgments

We specially thank Gabriela Nava and Miguel García from OCEANUS A.C. for all their help in the preparation and execution of the sampling campaigns. We deeply appreciate all advices and comments of Dr. John Norenburg (Smithsonian National Museum of Natural History) on the project. We thank all the help and support of the CONANP through Felipe Ángel Omar Ortiz and Yadira Gómez (Sian Ka’an Biosphere Reserve) and David Sima Pati (Cakalul Biosphere Reserve). We also acknowledge the support provided by the wildlife guards on duty during periods of collections. Invaluable help and support of Alejandro Tuz Novelo, Jorge Cruz Medina, Simon Trautwein, Lucia Montoliu Elena and León Ibarra Garibay for the field work and sample processing is deeply appreciated. This research was supported by the National Council of Science and Technology (CONACyT-Mexico) through International Postdoctoral Fellowship for the Consolidation of Research Groups (234894 and 265817). Sampling Campaigns were founded by OCEANUS A.C. and the 2015 Edward & Phyliss Reed Fellowship for Copepod Research (Smithsonian National Museum of Natural History). The comparative material for COI from Mexico is part of the contributions of the Mexican Barcode of Life (MEXBOL) supported by the National Council of Science and Technology (CONACYT, 271108). This publication has the number 60 from the Senckenberg am Meer Metabarcoding and Molecular Laboratory and the number 41 that uses data from the Senckenberg am Meer Conocal Laser Scanning Microscopy Facility. Comments and suggestions made by an anonymous reviewer and editors have improved the quality of our MS.

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Electronic Supplement Files
at http://www.senckenberg.de/arthropod-systematics


File 2: mercado&al-mastigodiaptomus-asp2018-electronicsupplement-2.pdf — COI phylogram of Mastigodiaptomus species, 118 specimens (sequenced in this study and downloaded from GenBank) based on Bayesian analyses. Nodal supports indicate posterior probabilities. Colors show the different localities following the haplotype network in figure 3.